# COMPARATIVE EVALUATION OF TISSUE RESPONSE OF MTA AND PORTLAND CEMENT WITH THREE RADIOPACIFYING AGENTS

*A Dissertation submitted*
*in partial fulfillment of the requirements*
*for the degree of*

## MASTER OF DENTAL SURGERY

**BRANCH – IV**

## CONSERVATIVE DENTISTRY AND ENDODONTICS


**THE TAMILNADU DR. MGR MEDICAL UNIVERSITY**

**CHENNAI – 600 032**

**2008 – 2011**

# Certificate

This is to certify that **Dr. M. HARIHARA SABARI,** post graduate student (2008 - 2011) in the Department of Conservative Dentistry and Endodontics, has done this dissertation titled "**COMPARATIVE EVALUATION OF TISSUE RESPONSE OF MTA AND PORTLAND CEMENT WITH THREE RADIOPACIFYING AGENTS"** under our direct guidance and supervision in partial fulfillment of the regulations laid down by **The Tamil Nadu Dr. M.G.R. Medical University, Guindy, Chennai – 32** for **M.D.S.** in Conservative Dentistry and Endodontics (Branch IV) Degree Examination.

**Dr. S. Jaikailash**
**Associate Professor**

**Dr. M. Kavitha**
**Professor & HOD i/c**
**Guide**

Department of Conservative Dentistry and Endodontics
Tamilnadu Government Dental College and Hospital
Chennai – 600 003.

**Dr. K.S.GA. NASSER**
**PRINCIPAL**

Tamilnadu Government Dental College and Hospital
Chennai – 600 003.

## ACKNOWLEDGEMENT

I wish to place on record my deep sense of gratitude to my mentor **Dr. M. KAVITHA, MDS.,** for the keen interest, inspiration, immense help and expert guidance throughout the course of this study as professor & HOD I/C & Guide of the Dept. of Conservative Dentistry and Endodontics, Tamilnadu Govt. Dental College and Hospital, Chennai.

It is my immense pleasure to utilize this opportunity to show my heartfelt gratitude and sincere thanks to **Dr.S. JAIKAILASH,MDS.,D.N.B.,** Associate Professor of the Department of Conservative Dentistry and Endodontics, Tamilnadu Govt. Dental College and Hospital, Chennai for his guidance, suggestions, source of inspiration and for the betterment of this dissertation.

I take this opportunity to convey my everlasting thanks and sincere gratitude to **Dr. K.S.G.A. NASSER, MDS.,** Principal, Tamilnadu Government Dental College and Hospital, Chennai for permitting me to utilize the available facilities in this institution.

I am extremely grateful to **Dr.C.S. ANANDALAKSHMI, Special Veterinary Officer, Animal Experimental Laboratory, Madras Medical College, Chennai – 3** for her guidance, suggestions and unconditional support to all my needs

made this study feasible. I extend my thanks to all the staff members of Animal Experimental Laboratory, Madras Medical College.

My sincere thanks to **Dr. Balachandran. M.V.Sc., Ph.D., Professor & HOD** and **Dr. Pazhanivel. M.V.Sc., Ph.D., Associate Professor,** Department of Veterinary Pathology, Madras Veterinary College, Chennai – 7 for their guidance, suggestions and tireless help, patience in carrying out the histological analysis.

I sincerely thank **Dr. B. Rama Prabha, MDS., Dr. K. Amudha Lakshmi, MDS., Dr. G. Vinodh, MDS., Dr. D. Aruna Raj, MDS., Dr.Nandhini. M.D.S., and Dr. Shakunthala. M.D.S.,** Assistant Professors for their suggestions, encouragement and guidance throughout this study.

I specially thank, **Dr.S.Porchelvan** B.Sc, MBA, PhD, Data manager, Biostatistician for all his statistical guidance and help.

I am more indebted to my beloved mother and grand mother for their continuous support and encouragement in every step that I take in my life.

I am always grateful to God Almighty, who is always beside me.

## DECLARATION

| | |
|---|---|
| TITLE OF DISSERTATION | **COMPARATIVE EVALUATION OF TISSUE RESPONSE OF MTA AND PORTLAND CEMENT WITH THREE RADIOPACIFYING AGENTS** |
| PLACE OF THE STUDY | Tamil Nadu Government Dental College & Hospital, Chennai – 3. |
| DURATION OF THE COURSE | 3 YEARS |
| NAME OF THE GUIDE | DR. M. KAVITHA. |
| HEAD OF THE DEPARTMENT | DR. M. KAVITHA |

I here by declare that no part of dissertation will be utilized for gaining financial assistance or any promotion without obtaining prior permission of the Principal, Tamil Nadu Government Dental College & HospitaL, Chennai – 3. In addition I declare that no part of this work will be published either in print or in electronic media without the guide who has been actively involved in dissertation. The author has the right to preserve for publish of the work solely with the prior permission of Principal, Tamil Nadu Government Dental College & Hospital, Chennai – 3.

**HOD i/c** **GUIDE** **Signature of the Candidate**

## CERTIFICATE

This is to certify that **Dr. M. Harihara Sabari**, Post Graduate Student, Tamil Nadu Government Dental College, Chennai – 3 had submitted his protocol (Part B Application) Vide 5/243/CPCSEA for the Dissertation Programme to the Animal Ethical clearance committee, Madras Medical College, Chennai – 3.

**TITLE:**

**COMPARATIVE EVALUATION OF TISSUE RESPONSE OF MTA AND PORTLAND CEMENT WITH THREE RADIOPACIFYING AGENTS.**

The Animal Ethical Clearance Committee experts screened his proposal 5/243/CPCSEA and have given clearance in the meeting held on 28.01.2010 at Dean's Chamber in Madras Medical College.

C. S. Anandalakshmi

**Dr. C. S. ANANDALAKSHMI.**
**Special Veterinary Officer.**
**Madras Medical College.**

SPECIAL VETERINARY OFFICER
ANIMAL EXPERIMENTAL LABORATORY
MADRAS MEDICAL COLLEGE
CHENNAI

**INSTITUTIONAL ETHICAL COMMITTEE**

**Tamil Nadu Government Dental College and Hospital,Chennai.3**
Telephone No: 044 2534 0343
Fax : 044 2530 0681

R.C No. 0427/DE/2010 Date: 18-03-2010.

Title of the work : Evaluation of tissue response to MTA and Portland cement with three radiopacifying agents.

Principal Investigator: Dr.M.Harihara Sabari, IInd Year MDS student,

Department : Dept of Conservative Dentistry & Endodontics, Tamil Nadu Govt. Dental College and Hospital,Chennai-3.

The request for an approval from the Institutional Ethical Committee (IEC) was considered for the following on the IEC meeting held on 18.01.2010 at the Principal's Chambers, Tamil Nadu Government Dental College & Hospital, Chennai-3.

"To get the Approval of the animal ethical committee at MMC for the animal study and proceed with the study".

The Members of the Committee, the Secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the Principal Investigator.

The Principal Investigator and their team are directed to adhere the guidelines given below:

1. You should get detailed informed consent from the patients/participants and maintain confidentiality.
2. You should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution or Government.
3. You should inform the IEC in case of any change of study procedure, site and investigation or guide.
4. You should not deviate from the area of work for which you have applied for ethical clearance.
5. You should inform the IEC immediately in case of any adverse events or serious adverse reactions. You should abide to the rules and regulations of the Institution.
6. You should complete the work within the specific period and if any extension of time is required, you should apply for permission again and do the work.
7. You should submit the summary of the work to the ethical committee on completion of the work.
8. You should not claim funds from the Institution while doing the work or on completion.
9. You should understand that the members of IEC have the right to monitor the work with prior intimation.
10. Your work should be carried out under the direct supervision of your Guide/Professor.

S Jayachandran 19/3/10
SECRETARY

18/3/10
CHAIRMAN

## CONTENTS

# INTRODUCTION

The aim of the endodontic treatment is to clean, disinfect and seal the root canal system. Nevertheless, in some cases, due to the complex anatomy or iatrogenic procedures, it is not possible to reach this goal. In some cases, treatment failure is solved by endodontic surgery. Periapical surgery usually consists of apicoectomy, apical cavity preparation and root end filling to seal the communication pathways between the root canal system and periapical tissues.

For a long time, the materials of choice for this procedure have been amalgam, IRM, Super-EBA and glass ionomer cements. However, these materials have the disadvantages of undergoing corrosion, electrolysis, delayed expansion and staining (amalgam), marginal leakage, moisture sensitivity and toxicity for vital tissues[43].

MTA (Pro Root MTA, Dentsply Tulsa, U.S.A.) basically composed of Portland cement 75% by weight, gypsum 5% by weight and bismuth oxide 20% by weight. The major component Portland cement is a mixture of dicalcium silicate, tricalcium silicate, tricalcium aluminate. Bismuth oxide added to provide radiopacity greater than dentin.

Sealing ability of MTA found to be superior than amalgam and super EBA and IRM[55,37]. MTA exhibits acceptable in vivo biologic performance when used for root-end fillings, perforation repairs, pulp capping and pulpotomy, and apexification treatment[58,59]. MTA induces biomineralization of cementoblasts[24] and stimulate mineralization[30].

Portland cement is the most common type of cement in general use around the world. Type I Portland cement is the main component of MTA with addition of bismuth oxide at 4:1 ratio to provide radiopacity. Comparative chemical study and X-ray diffraction analysis of MTA and Portland cements proved that Portland cement is similar to MTA with the exception of Bismuth oxide which is present only in MTA[40,26]. Histologic evaluation studies showed that Portland cement showed similar inflammatory results when compared with MTA[45,11,54]. Portland cement also proved to be comparable with MTA in hard tissue formation when used as direct pulp capping material still maintaining pulp vitality[46,3,53]. Regular and white Portland cements are biocompatible, do not induce cellular death and have antimicrobial activity[17,41].

Portland cement does not have sufficient radiopacity to be visualised radiographically and thus a radiopacifying agent must be added to its composition. Bismuth oxide 20% is the radiopacifier present in MTA, atleast 15% of bismuth oxide to be added to white Portland cement to provide sufficient radiopacity[10]. However, it is questioned if bismuth oxide would be the best radiopacifying agent to be associated with Portland cement. The addition of bismuth oxide radiopacifier decreased mechanical stability by introducing flaws and increased porosity[15]. Saliba et al showed that addition of bismuth oxide did not affect the compressive strength of Portland cement[49]. There is a need to search for an alternative radiopacifying agent to be associated with Portland cement.

The ISO 6876/2001 standard established that root canal sealers should be at least as radiopaque as 3mmAl. According to the American National Standards Institute and American Dental Association Specification No.57, endodontic filling materials should present a difference in radiopacity equivalent to at least 2mmAl in comparison to bone or dentin. Materials like bismuth carbonate, iodoform, zirconium dioxide, barium sulphate, bismuth subnitrate, had radiopacity values above that of dentin and the minimum recommended by the ANSI/ADA can be used as radiopacifiers. The possible interference of the radiopacifiers with biocompatibility of Portland cement should be investigated[39]. Iodoform 20wt% added with Portland cement showed similar tissue response as MTA, in a rat subcutaneous tissue implantation study[11].

The implantation of materials in to connective tissue of small animals is considered a suitable secondary test (local toxicity) for the evaluation of the biocompatibility of endodontic materials[18]. The subcutaneous implantation method used in this study is a practical method for the qualitative evaluation of endodontic materials, and can yield exact detailed information about material-tissue reaction on the cellular level[6].

The objective of this study is to evaluate the biocompatibility of White Portland cement 80wt% mixed with three radiopacifying agents - 20wt% (bismuth oxide/ iodoform / zirconium dioxide) and compared with MTA(Pro Root MTA).

## AIMS AND OBJECTIVES

The aim of the study was to compare the tissue reaction of white Portland cement(WPC) (80wt%) mixed with (20wt%) radiopacifying agents: Bismuth oxide/Iodoform/zirconium dioxide with MTA (Pro Root MTA) in rat subcutaneous connective tissue by light microscopic histological evaluation.

The objectives were:

i. To mix WPC 80wt% with Bismuth oxide 20wt%, WPC 80wt% with Iodoform 20wt%, and WPC 80wt% with 20wt% Zirconium dioxide.

ii. Subcutaneous implantation of the above materials and MTA loaded in polyethylene tube in white albino rat.

iii. Histopathological evaluation of the subcutaneous tissue along with the tube by light microscopy after the experimental periods 7, 30 and 60 days.

iv. To compare the tissue reaction of the materials individually with each other.

# REVIEW OF LITERATURE

## MINERAL TRIOXIDE AGGREGATE (MTA):

Mineral Trioxide Aggregate (MTA) was a biomaterial that has been investigated for endodontic applications since the early 1990s. Originally developed by Torabinejad at Loma Linda university. MTA was first described in the dental scientific literature in 1993 and was given approval for endodontic use by the U.S. Food and Drug Administration in 1998.

**Torabinejad et al (1995)**[35] determined the chemical composition, pH, compressive strength and radiopacity of MTA. He showed that the main molecules present in MTA are calcium and phosphorus ions. In addition, MTA had a pH of 10.2 initially which then rised to 12.5 three hours after mixing. MTA was more radiopaque than Super EBA and IRM. MTA had a longer setting time of 2 hours and 45 minutes. At 24 hours MTA had the lower compressive strength of 40MPa but it increased after 21 days to 67 MPa.

**Torabinajed et al (1995)**[36] showed that the tissue reaction to MTA implantation in the mandible of guinea pig was milder than that observed with Super EBA implantation. It seemed that Super EBA and MTA were biocompatible.

**Torabinajed et al (1995)**[37] proved that MTA provided better adaptation seal than commonly used root end filling materials such as amalgam, Super EBA and IRM.

**Eng Tiong Koh et al (1998)**[20] proved that the ELISA assays revealed raised levels of all Interleukins at all periods when cells were grown in the presence of MTA; in contrast, cells grown alone or with IRM produced undetectable amounts. The macrophage colony stimulating factor was produced by cells irrespective of the group. It seemed that MTA offers a biologically active substrate for bone cells and stimulated interleukin production.

**Holland R et al (1999)**[25] theorized that the tricalcium oxide in MTA reacted with tissue fluids to form calcium hydroxide, resulting in hard-tissue formation in a manner similar to that of calcium hydroxide.

Compared with calcium hydroxide, MTA had demonstrated a greater ability to maintain the integrity of pulp tissue. **Aeinehchi et al (2003)**[1] showed that histologic evaluation of pulpal tissue in animals and humans demonstrated that MTA produced a thicker dentinal bridge, less inflammation, less hyperemia and less pulpal necrosis compared with calcium hydroxide.MTA also appeared to induce the formation of a dentin bridge at a faster rate than did calcium hydroxide. The process by which MTA acted to induce dentin bridge formation, however, is not known.

In 2002, in addition to the traditional gray MTA, White MTA was introduced. **Saeed Asgary et al (2005)**[52] concluded that concentrations of $Al_2O_3$ , MgO, and particularly FeO in WMTA was considerably lower than those found in GMTA. Differences in the observed FeO concentration were thought

to be primarily responsible for the variation in color of the WMTA in comparison with GMTA.

**Sarkar et al (2005)**[51] showed that MTA materials were a mixture of a refined Portland cement and Bismuth oxide as radiopacifier and trace amounts of $SiO_2$, CaO, MgO, $K_2SO_4$, AND $Na_2SO_4$. The major component of Portland cement was a mixture of dicalcium silicate, tricalcium silicate, tricalcium aluminate, gypsum and tetracalcium aluminoferrite.

**Camilleri (2006)**[9] concluded that MTA materials formed a colloidal gel that solidifies to a hard structure in approximately 3-4 h. Hydrated MTA products had an initial pH of 10.2 which rises to 12.5 three hours after mixing. The setting process was described as a hydration reaction of tricalcium silicate and dicalcium silicate, similar to its parent compound Portland cement that needed sufficient water for reaction to occur.

MTA has a wide clinical application. **Peng et al(2006)**[44] showed that in primary molar teeth with vital pulp exposure caused by caries or trauma, a pulpotomy performed with MTA results in better clinically and radiographically observed outcomes. Fewer undesirable responses were recorded for MTA than when formocresol was used.

**Ahmed et al (2008)**[4] showed that Pro Root MTA has excellent sealing ability and could be used with or without matrix in repair of large furcation perforations and the use of IRM to repair large furcation perforations should be limited.

**Witherspoon et al (2008)**[58] showed that MTA obturation of canals with open apices was a viable alternative to the use of $Ca(OH)_2$ to induce apical closure.

**William Saunders et al (2008)**[59] in his prospective clinical study of periradicular surgery concluded that MTA as a root end filling material showed a high success rate when compared with others.

**Sema S. Hakki et al (2009)**[50] concluded that MTA did not have a negative effect on the cell survival and morphology of cementoblasts but MTA induced biomineralisation of cementoblasts.

## MTA and PORTLAND CEMENT

**Jacob Saidon et al (2003)**[31] compared the in vitro cytotoxic effect of MTA and Portland cement in L929 cells and tissue reactions of both the materials in bone implantation in the mandibles of guinea pigs. There was no difference in cell reactions in vitro. Bone healing and minimal inflammatory response adjacent to ProRoot and Portland cement were observed, suggesting both materials were well tolerated. He concluded that MTA and Portland cement show comparative biocompatibility when evaluated in vitro and in vivo.

**Razmi et al (2004)**[45] evaluated the tissue reaction to implanted MTA and Portland cement in the mandible of cats. The physical and histological results observed with MTA were similar to those of Portland cement. Both the materials were considered biocompatible.

**Renato Menezes et al (2004)**[46] investigated the pulpal response of dogs' teeth after pulpotomy and direct pulp protection with MTA Angelus, ProRoot, Portland cement and WPC. All the materials demonstrated similar results when used as pulp capping materials. Pulp vitality was maintained in all specimens and the pulp had healed with a hard tissue bridge. The study concluded that Portland cement and MTA were equally effective as pulp protection materials following pulpotomy.

**Durate et al (2005)**[38] concluded that the release of arsenic from Portland cement and MTA were similar and were well below those considered to be harmful.

**Islam et al (2005)**[26] compared the major constituents present in ProRoot MTA, ProRoot MTA(tooth coloured) and ordinary Portland cement and white Portland cement using powder X-ray diffractometery. The main constituents were found to be tricalcium silicate, tricalcium aluminate, dicalcium silicate and tetracalcium aluminium ferrite in all the four cements with the additional presence of $Bi_2O_3$ in Pro Root MTA and Pro Root MTA (tooth coloured).

**Daniel Araki Ribeiro (2005)**[17] evaluated the genotoxic and cytotoxic effects of MTA and Portland cements in vitro using the alkaline single cell gel (comet) assay and trypan blue exclusion test, respectively on mouse lymphoma cells. The results demonstrated that the single cell gel assay failed to detect DNA damage after a treatment of cells by MTA and Portland cement. The study concluded that none of the compound tested were cytotoxic.

**Marilia Gerhardt de Oliveira et al (2007)**[34] analyzed and compared Portland cement with MTA. Similar chemical elements were found in all materials and there was a small percentile variation among them. Bismuth was detected only in MTA composition. In spite of the chemical similarity, it was observed a difference in the texture and in the particles of each material. Pro Root MTA presented the highest percentage of bismuth (9.2% on average). Except for bismuth, Portland cement and MTA presented similar chemical formulations.

**De Deus et al (2007)**[16] compared the sealing ability of four hydraulic cements, including Pro Root MTA and Portland cement. He concluded that no cement was capable of producing a fluid tight seal and the sealing ability promoted by MTA and Portland cement were similar.

**Augusto Bodanezi et al (2008)**[5] investigated the solubility of mineral trioxide aggregate and Portland cement. Only Portland cement showed less than 3% weight loss through 24 hours. Detached MTA residues were heavier than those of Portland cement over the 3 to 168 hours. The study concluded that in an aqueous environment MTA was more soluble than Portland cement and exceeds the maximum weight loss considered acceptable by ISO 6876 (2001).

**Bramante (2008)**[7] analysed the concentration of arsenic in Portland cement and MTA. He concluded that the concentrations were well below the limit set in ISO 9917-1.

**Amir Shayegan et al (2009)**[2] compared the response of the pulp of primary pig teeth after capping with beta-tricalcium phosphate, white MTA, white Portland cement and calcium hydroxide. There was no significant difference between the materials in terms of primary pulp response, hard tissue formation and normal pulp tissue preservation. Beta-tricalcium phosphate, WMTA and White Portland cement in primary pig teeth were as effective as $Ca(OH)_2$ in pulp capping.

**Taisa Regina Conti et al (2009)**[53] reported two clinical cases in which Portland cement was applied as a medicament after pulpotomy of mandibular primary molars. At the 12 month follow up , clinical and radiographic examinations of the pulpotomized teeth and their periradicular area revealed that the treatments were successful in maintaining the teeth asymptomatic, preserving pulp vitality and formation of a dentin bridge immediately below the Portland cement.

**Antonio Vinicius Holanda Barbosa et al (2009)**[3] evaluated the short term response of human pulp tissue when directly capped with Portland cement. Portland cement exhibited some features of biocompatibility and capability of inducing mineral pulp response in short term evaluation. The results suggested that the Portland cement had a potential to be used as a less expensive pulp capping material in comparison to other pulp capping materials.

## RADIOPACIFYING AGENTS WITH PORTLAND CEMENT

**Coomaraswamy et al (2007)**[15] investigated the effect of bismuth oxide radioopacifier addition on the material properties of an endodontic Portland cement based system. The study concluded that the addition of $Bi_2O_3$ radioopacifier decreased mechanical stability by introducing flaws and increased porosity by leaving more unreacted water within the Portland cement. Flaws in the set cement matrix might exacerbate existing cracks ; moreover increased porosity is known to increase the solubility and thus the degradation of the material. This might potentially affect the longevity of the material, compared to that of pure Portland cement, because the set material was more likely to degrade and was thus more likely to be compromised as a sealant.

**Camilleri (2007)**[9] stated that the addition of bismuth oxide to MTA had been shown to affect the hydration mechanism of MTA. It forms part of the structure of calcium silicate hydrate, which was the main by product of cement hydration and also affects the precipitation of calcium hydroxide in the hydrated paste.

**Marco Antonio Hungaro Durate et al (2008)**[11] evaluated the radiopacity of Portland cement associated with the following radiopacifying agents: bismuth oxide, zinc oxide, lead oxide, bismuth subnitrate, bismuth carbonate, barium sulphate, iodoform, calcium tungstate, and zirconium oxide. A ratio of 20% radiopacifier and 80% white Portland cement by weight was used for analysis. The study concluded that radiopacity of pure Portland cement

was significantly lower than that of dentin. All the materials evaluated in the study had radiopacity values above that of dentin and the minimum recommended by ANSI/ADA.

**Carlos Eduardo da Silveira Bueno et al (2009)[10]** determined the ideal concentration of bismuth oxide in white Portland cement to provide it with sufficient radiopacity for use as an endodontic material.(ADA specification #57). The readings of MTA and white Portland cement with 15% bismuth oxide did not differ significantly from the reading observed for a thickness of 4mm of aluminium, which is considered ideal. White MTA and white Portland cement with 15% bismuth oxide presented the radiopacity required for an endodontic cement.

**Saliba E et al (2009)[49]** evaluated the strength and radiopacity of Portland cement with varying additions of bismuth oxide. He concluded that the addition of bismuth oxide did not seem to affect the compressive strength of Portland cement. All the bismuth oxide (10% to 30%) replaced cements had radiopacities higher than 3mm thickness of aluminium.

**Yun Chan Hwang et al (2009)[63]** compared the chemical constitution, radiopacity, and biocompatibility of Portland cement containing bismuth oxide with those of Portland cement and MTA. The chemical constitution was determined by energy-dispersive X ray analysis (EDX) attached to a scanning electron microscope. Cytotoxicity was evaluated using MTT assay. Tissue reaction was studied by subcutaneous implantation of the materials loaded in

polyethylene tubes in the dorsal region of rats. The study concluded that the constitution of all materials were similar. However, the Portland cement were more irregular and had a larger particle size than MTA. The MTT assay revealed MTA to have slightly higher cell viability than the other materials. There was no significant difference in the tissue reaction between the experimental groups.

**Camilleri (2009)[14]** investigated the physical and chemical properties of Portland cement loaded with alternative radiopacifying materials (barium sulphate, gold and silver/tin alloy) for use as root end filling materials in a mineral trioxide aggregate like system. It was concluded that the bismuth oxide in MTA could be replaced by gold and silver/tin alloy. The physical, mechanical and chemical properties of the cement replaced with alternative radiopacifiers were similar and comparable to ProRoot MTA.

The ISO 6876/2001 standard establishes that root canal sealers should be at least as radiopaque as 3 mmAl. According to the American National Standards Institute and American Dental Association specification No.57, endodontic filling materials should present a difference in radiopacity equivalent to at least 2 mmAl in comparison to bone or dentin.

## SUBCUTANEOUS IMPLANTATION

**Torneck et al (1966)[12]** evaluated the polyethylene tubes of varying length and diameter by implanting them in the dorsal subcutaneous tissues of

wistor rats. Length of the tube used were 4mm, 6mm and 10mm. Inside diameter of the tube were 0.58mm, 0.86mm, 1.14mm and 1.40mm. The absence of inflammation in the connective tissue encapsulating the polyethylene implants indicated the acceptability of the material for test purposes.This capsule formation occurred as a result of the displacement of the connective tissue fascia and the proliferation of connective tissue elements about the implanted tubes.

**Langeland et al (1969)**[33] compared the methods used to evaluate the biologic responses to endodontic material. The study concluded that the implantation test may be used only as a short term preliminary screening test, but tests in teeth would have to be performed for the decisive evaluation. When used as a preliminary screening test, placement of the test material in polyethylene tubes control the quantity and form and prevent the material from major disintegration, eliminating these variables.

**Olsson et al (1981)**[6] concluded that the subcutaneous implantation method possessed several of the qualities desired of a secondary test for the biologic evaluation of endodontic materials. The subcutaneous implantation method was a practical method for the qualitative evaluation of endodontic materials, and could yield exact detailed information about material-tissue reaction on the cellular level.

**Carlos Alberto Herrero de Morais et al (2006)**[11] evaluated the biocompatibility of Portland cement with the addition of iodoform, compared to

MTA(Pro Root). 20% iodoform was used. The materials were mixed and filled in polyethylene tubes and then subcutaneously implanted in albino rats. The study concluded that there were no difference between inflammatory responses at 7 and 30 days. After 60 days there was significantly more tissue reaction to MTA and Portland cement plus iodoform when compared with empty polyethylene tubes.

**Tauby Coutino Filho et al (2009)**[54] evaluated the subcutaneous connective tissue reactions in albino rats and the radiopacity of MTA, Portland cement, and Portland cement plus bismuth oxide. No difference were found for the tissue response between Portland cement and MTA. A positive correlation between bismuth oxide and radio opacity of Portland cement was determined.

# MATERIALS AND METHODS

## MATERIALS USED:

Pro Root MTA (Dentsply, U.S.)

Birla White cement (Grasim Ind Ltd. Aditya Birla group)

Bismuth Oxide LR (Chen Chemicals, India)

Iodoform (Vikash Pharma, India)

Zirconium dioxide (Lobal Ltd, India)

## ANIMAL USED:

Rattus norvegicus – white albino rats 18 in numbers.

## ARMAMENTARIUM

- Polyethylene tubes (1.2 mm X 45mm) (B.D. Venflon, Becton Dickinson Ind (P) Ltd.)
- Ketamine HCl (Aneket)
- Metal scale
- Super max shaving blade
- B.P. Handle
- B.P. Blade No. 15
- B.P. Blade No. 11
- Haemostat
- Tweezer

- 2 ml disposal syringe
- Betadine
- 3-0 silk suture (Trusilk sutures India Ltd)
- Glass slab
- Cement spatula
- Stainless steel containers – 5 numbers (6 X 6 X 4cms)
- Stainless steel tray
- Cotton
- 10% formalin
- Sterilized containers – 90 numbers for specimen collection.

## MATERIALS

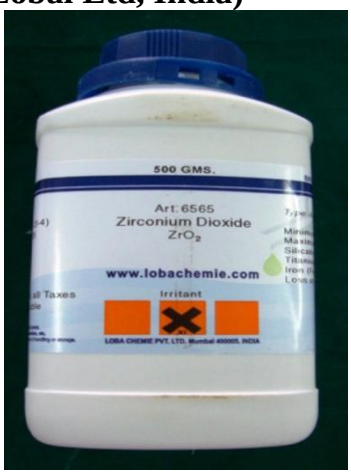
**Fig. 1 : Pro Root MTA (DENTSPLY, TULSA, USA)**


**Fig. 2 White Portland Cement Birla White (Grasim Ind Ltd., Aditya Birla Group)**


**Fig. 3 Bismuth Oxide (Chen Chemicals, India)**


**Fig. 4 Iodoform (Vikas Pharma, India)**


**Fig. 5 Zirconium dioxide (Lobal Ltd, India)**



**EXPERIMENTAL GROUPS:**

GROUP I - EMPTY POLYETHYLENE TUBE

GROUP II - MTA

GROUP III - WHITE PORTLAND CEMENT 80wt% + BISMUTHOXIDE 20wt%

GROUP IV - WHITE PORTLAND CEMENT 80wt% + IODOFORM 20wt%

GROUP V - WHITE PORTLAND CEMENT 80wt% + ZIRCONIUM DIOXIDE 20wt%

**EXPERIMENTAL PROTOCOL:**

Phase I – MATERIAL PREPARATION

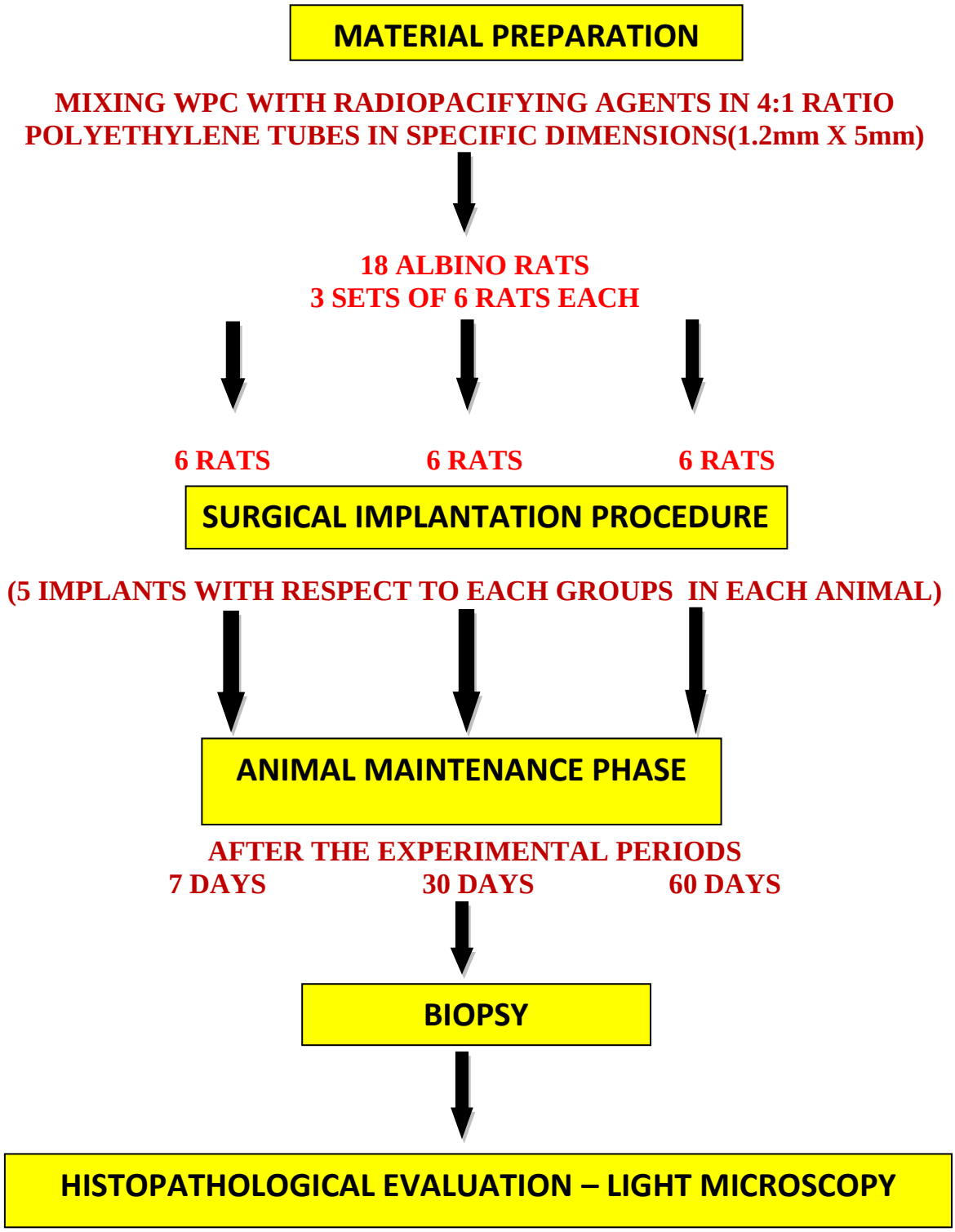
Phase II – SURGICAL IMPLANTATION PROCEDURE

Phase III – ANIMAL MAINTENANCE PHASE TILL THE RESPECTIVE EXPERIMENTAL PERIODS

Phase IV – BIOPSY & SACRIFICE OF THE ANIMAL AFTER EXPERIMENTAL PERIODS

Phase V – HISTOPATHOLOGICAL EVALUATION

## FLOW CHART


MATERIAL PREPARATION
MIXING WPC WITH RADIOPACIFYING AGENTS IN 4:1 RATIO
POLYETHYLENE TUBES IN SPECIFIC DIMENSIONS(1.2mm X 5mm)
18 ALBINO RATS
3 SETS OF 6 RATS EACH
6 RATS
6 RATS
6 RATS
SURGICAL IMPLANTATION PROCEDURE
(5 IMPLANTS WITH RESPECT TO EACH GROUPS IN EACH ANIMAL)
ANIMAL MAINTENANCE PHASE
AFTER THE EXPERIMENTAL PERIODS
7 DAYS
30 DAYS
60 DAYS
BIOPSY
HISTOPATHOLOGICAL EVALUATION – LIGHT MICROSCOPY

## SURGICAL IMPLANTATION PROCEDURE

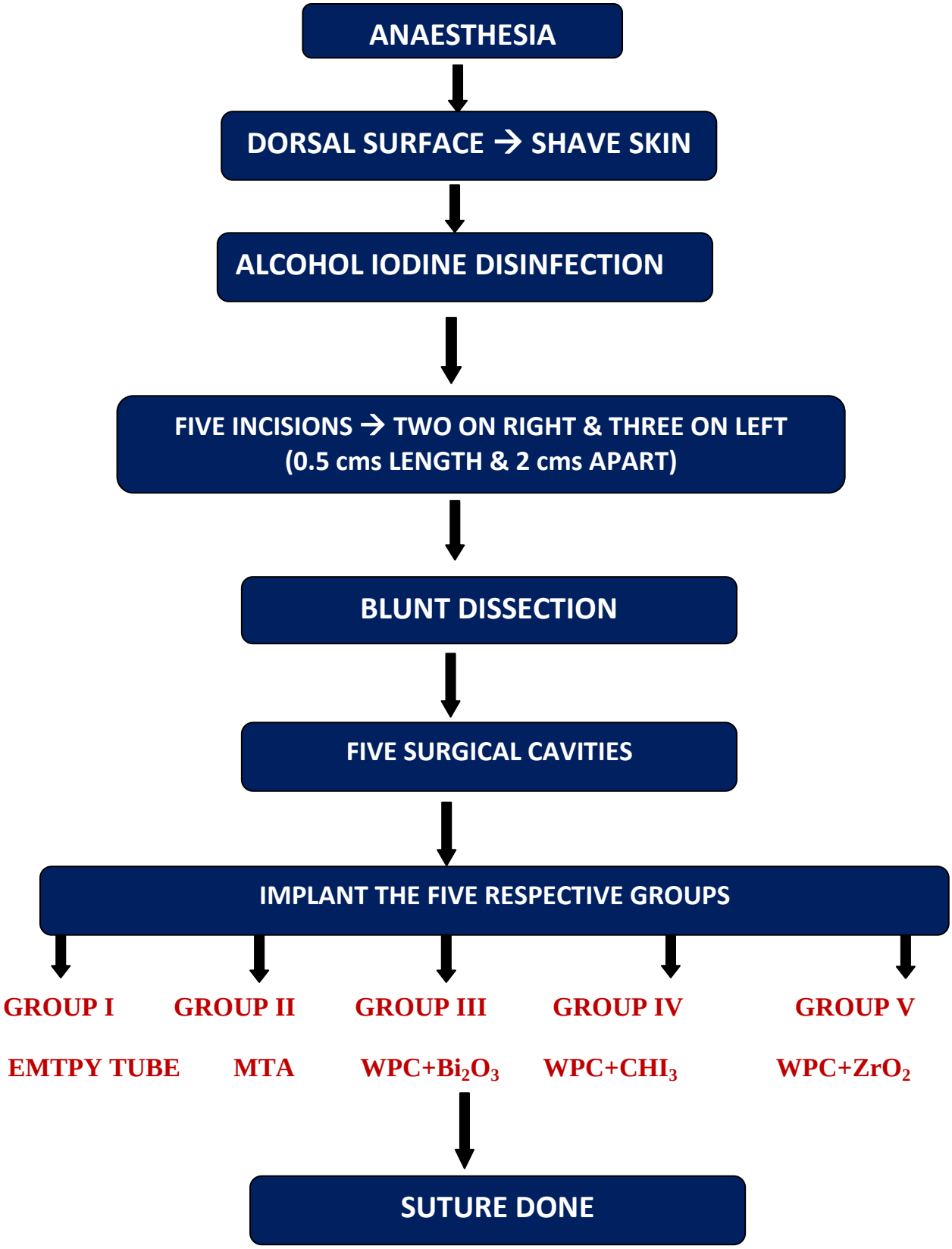
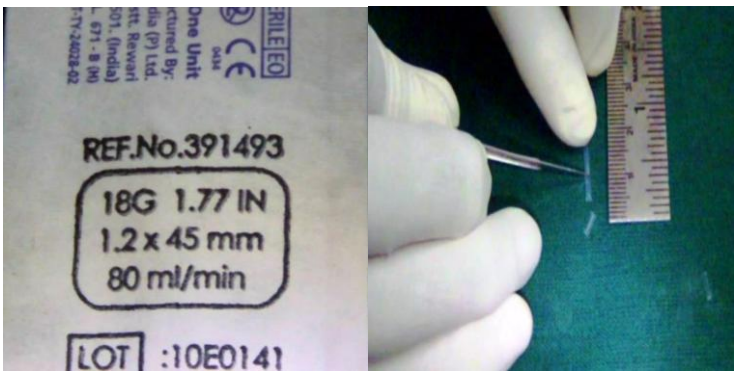


**Fig. 6 Armamentarium for Surgical Procedure**

**Fig. 7 Weighing machine**

**Fig. 8 B.D. Venflon I. V. Infusion apparatu with polyethylene tube of 1.2mm diameter cut into 5mm lengths**




.

## METHODOLOGY:

The study has been approved by the Ethical Committee of Tamil Nadu Government Dental College, Chennai -3 and the Animal Ethical Committee of Madras Medical College, Chennai.

18 male wistor albino rats of 5 – 6 months old each weighing 200 ± 25gms were used in this study. 18 animals were divided into 3 sets of 6 each for the respective experimental periods - 7 days, 30 days and 60 days. All the animals were housed at the Animal Department of Madras Medical College, Chennai and were fed appropriate granular food and water ad libitum.

## MATERIAL PREPARATION:

A total of 90 Polyethylene tubes of the desired dimension 1.2mm diameter and 5 mm length were made from new unopened B.D. Venflon intravenous apparatus.(Fig.8)

White Portland cement WPC (Birla White, Grasim Ind Ltd) is mixed with the radiopacifying agents in ratio of 4:1. In Group III, 80 wt% WPC is mixed with 20wt% Bismuth oxide(Chen chemicals, India). In Group IV, 80 wt% WPC is mixed with 20wt% Iodoform (Vikash Pharma, India). In Group V, 80 wt% WPC is mixed with 20wt% Zirconium dioxide (Lobal Ltd, India). All the materials were weighed properly and mixed in the desired ratio.(Fig. 7)

**SURGICAL IMPLANTATION PROCEDURE:**

90 Polyethylene tubes were autoclaved. 5 tubes were used in each animal. A empty polyethylene tube (Group I) implanted in each animal was used as the control. Group II MTA (Pro Root, Dentsply) was mixed with distilled water according to the manufacturer's instructions in the powder liquid ratio of 3:1.

The other three groups, Group III - 80wt% WPC mixed with 20wt% Bismuth oxide, Group IV – 80wt% WPC mixed with 20wt% Iodoform, Group V – 80wt% WPC mixed with 20wt% Zirconium dioxide were mixed with sterile saline in the powder liquid ratio of 3:1. A sterile glass slab and cement spatula was used to mix the materials. All the materials were carefully loaded into the polyethylene tube so that the tube is fully loaded. The tubes were implanted immediately after loaded with the test materials.

The animals were anaesthetized with Ketamine hydrochloride (Aneket) in all surgical periods. Ketamine 40 mg/kg body weight is used as recommended by Miami Univeristy, Lab animal anaesthesia[60]. Ketamine is given intramuscularly in the thigh muscle of the albino rat with onset of action within 5 minutes and duration lasting for 80 minutes.(Fig. 9) The dorsal skin was shaved carefully without injuring the skin of the animal. It was then disinfected with alcohol iodine solution.

A total of 5 implant on each animal two on the right side and three on the left side on the dorsal surface of the animal was decided. Five incisions were made on the back of the albino rat, 2cms from the spine. Incisions were made

over a length of 0.5cm using a No.15 B.P. blade in a head to tail alignment.(Fig.10) There should be atleast 2 cms distance between the incisions to prevent interaction of the materials. Five surgical pouches were created by blunt dissection, each for the respective groups (Fig. 11). The tubes that were previously loaded with the materials were implanted into the surgical cavities, parallel to the incisions, which could prevent dislodgement or loss of the implant till the experimental periods were over (Fig. 16). Care must be taken to prevent smearing of the material in the lateral side of the tubes. The position in which each group was implanted was standardized. Incision were then sutured with a 3-0 silk (Fig. 17) (Tru silk sutures Ind Ltd.). All surgical procedures were performed under supervision of the Veterinarian of the animal laboratory.

**MAINTENANCE PHASE:**

After the surgical procedure was over, the animals were observed until recuperation of their physical activities and placed in cages with no feeding restrictions. (Fig. 20,21). All the 18 animals irrespective of the experimental periods after the surgical procedure were placed in individual cages and were seen daily till the experimental periods - 7 days, 30 days and 60 days were over. Suture removal was done after 7 days.

**Fig. 9 I.M. Ketamine inj.**

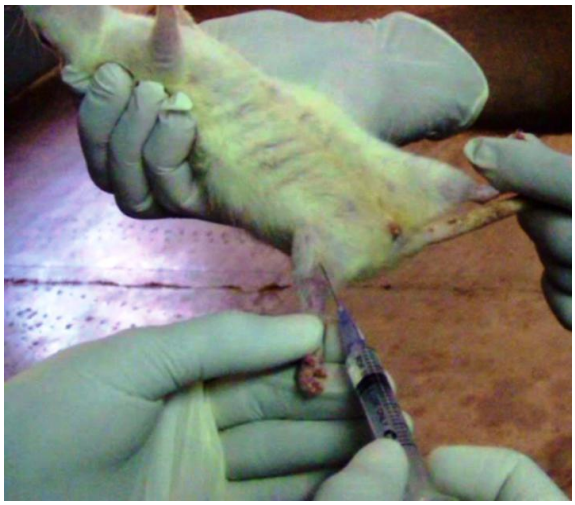


**Fig. 10 Incision using B.P Blade No.15**

**Fig. 11 Surgical cavity preparation**

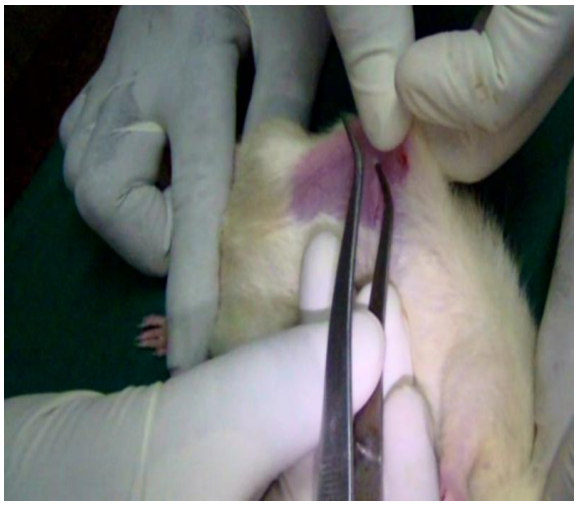


**Fig. 12 Placing EMPTY tube into The surgical cavity**

**Fig. 13 MTA and Polyethylene tube**

**Fig. 14 Packing MTA into the tube**

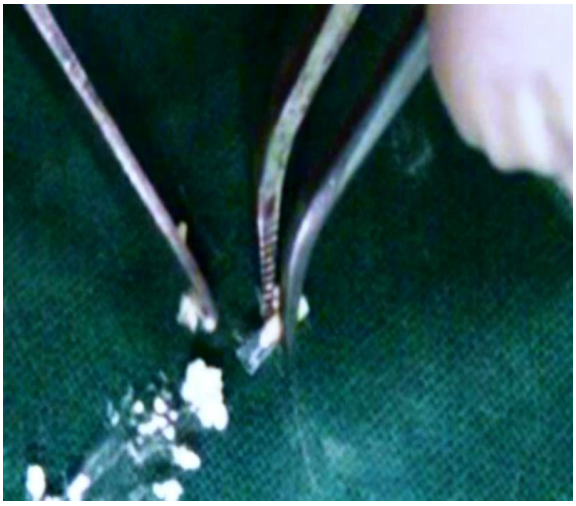


**Fig. 15 MTA packed polyethylene tube**

**Fig. 16 MTA filled tube placed into the prepared surgical cavity**

**Fig. 17 Sutured with 3-0 silk**

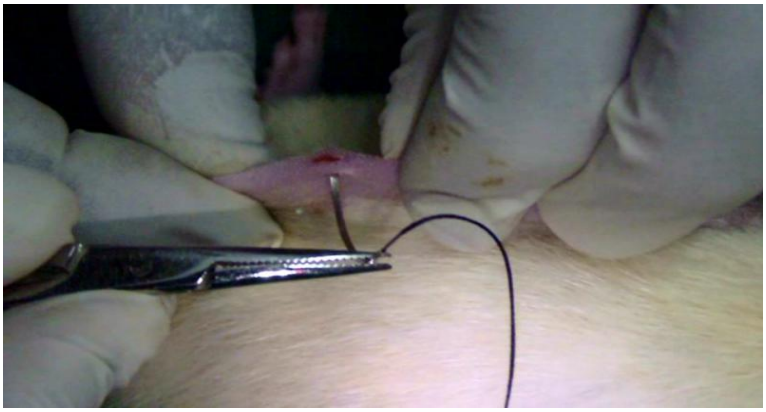


**Fig. 18 Two implants on right side  placed & sutured**

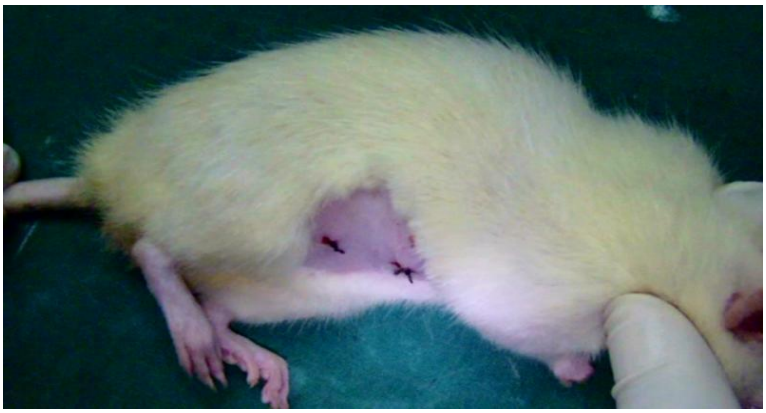


**Fig. 19 Three implants on left side placed & sutured**

**BIOPSY:**

After the respective experimental periods 7 days, 30 days and 60 days (6rats in each), the animals were again anaesthetized for excisional biopsy of the implant with the surrounding tissues. The animals were again anaesthetized by Ketamine hydrochloride as explained earlier for implantation procedure.

The full body of the animal was then taken a radiograph (Fig. 22,23). This will help to locate the implanted tube which were loaded with materials that were radiopaque. The empty tube which is radiolucent can also be easily located which was 2 cms from the adjacent implanted tube which was radiopaque. Even though the tube can be located by palpating the tube in the dorsal surface after shaving the area, radiograph acts as an adjunct in locating the implanted polyethylene tubes.

After anaesthesia and shaving, the located implanted tubes were carefully excised together with the surrounding connective tissue. 6 specimens in each group for each experimental periods. So 18 specimens for each group with a overall total of 90 specimens. All were subjected to histopathological evaluation. Animals were sacrificed by an overdose of anaesthetic immediately after removal of the tissue samples.

**HISTOPATHOLOGICAL ANALYSIS:**

The specimens were placed in 10% buffered formalin until histologically processed. Tissues were embedded in paraffin wax. The paraffin wax were

oriented parallel to the tube long axis. 4 µm longitudinal sections (including the open ends of the tubes) were obtained with a microtome to allow examination of the tissues in contact with the test materials. Sections were stained with hematoxylin and eosin (Leica Semiautomatic stainer) and viewed under light microscopy at X40, X100 and X200 magnification (BX 51 Olympus Multihead light microscope). The interface at the opening of the polyethylene tubes between the material and the tissue, was examined and evaluated for the intensity of inflammation. The occurrence of chronic inflammatory infiltrate composed of macrophages, lymphocytes and plasmocytes as well as the presence of eosinophils and multinucleate foreign body giant cells was evaluated at all evaluation periods. these sections were also evaluated for the occurrence and thickness of fibrous capsule. These inflammatory responses were scored according to the following criteria[47]

0 – no reaction (absence of inflammatory cells)

1 – mild reaction (presence of mild chronic inflammatory infiltrate)

2 – moderate reaction (presence of moderate chronic inflammatory infiltrate, or some eosionophils or giant cells)

3 – severe reaction (presence of an intense chronic inflammatory infiltrate, large number of eosinophils or giant cells)

The qualitative data were analysed using Pearson Chi – Square test in SPSS version 15. The significance was set at 5% for all analysis. Each group was compared individually with other groups.

**Fig. 20 Maintenance phase**
**Each rat maintained in individual cages**

**Fig. 21 Albino Rat in cage after surgery**

**Fig.22 Whole body of animal radiographed**

**Fig.23 Radiograph showing animal with implants**

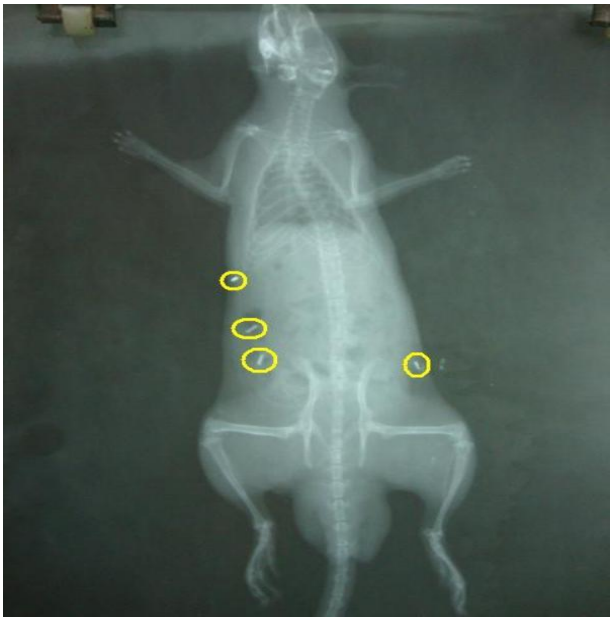


**Fig.24 Tissue samples**


**Fig. 25 Tissue samples in 10% formalin**


**Fig. 26 : TISSUE SAMPLE WITH EMTPTY POLYETHYLENE TUBE**

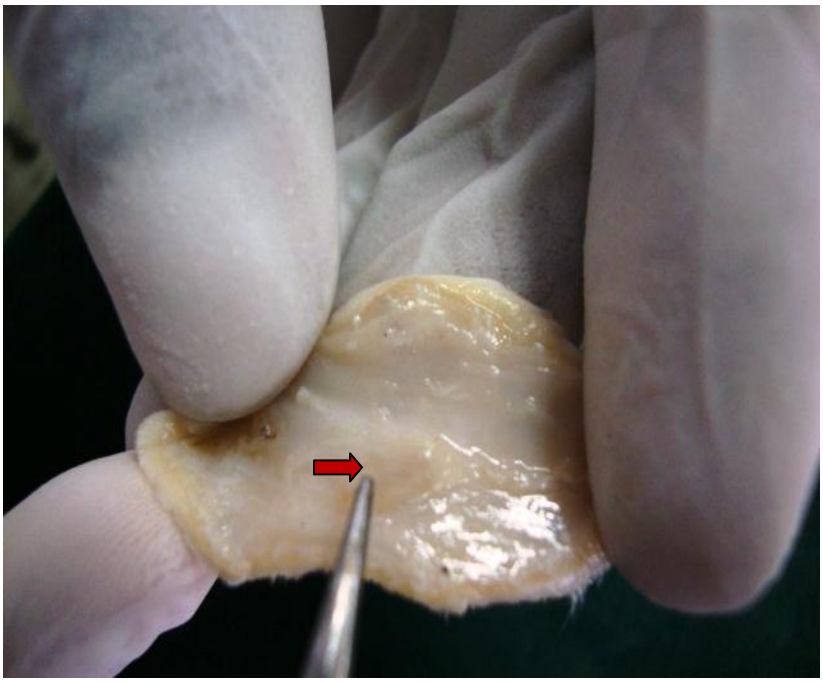


**Fig. 27 :TISSUE SAMPLE WITH MTA FILLED TUBE**

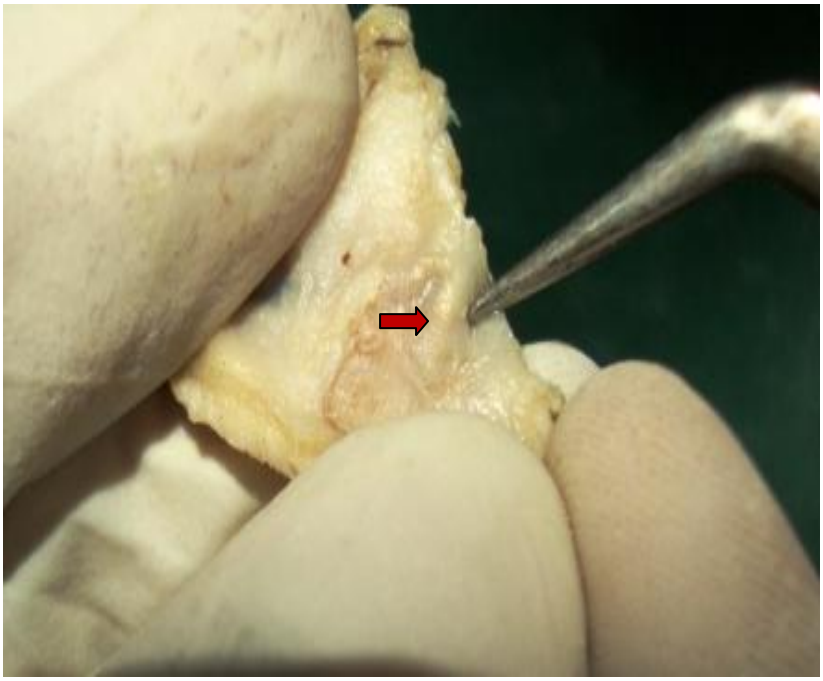


**Fig. 28: TISSUE SAMPLE WITH PC+$BI_2O_3$ FILLED POLYETHYLENE TUBE**

**Fig. 29. TISUE SAMPLE WITH PC+IODOFORM FILLED POLYETHYLENE TUBE**

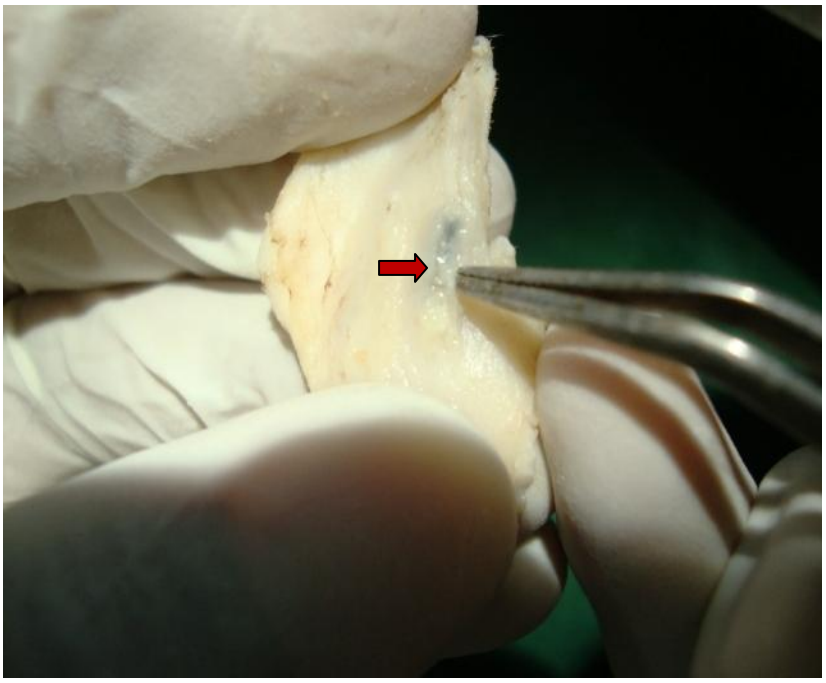


**Fig. 30. TISSUE SAMPLE WITH PC+$ZrO_2$ FILLED POLYETHYLENE TUBE**

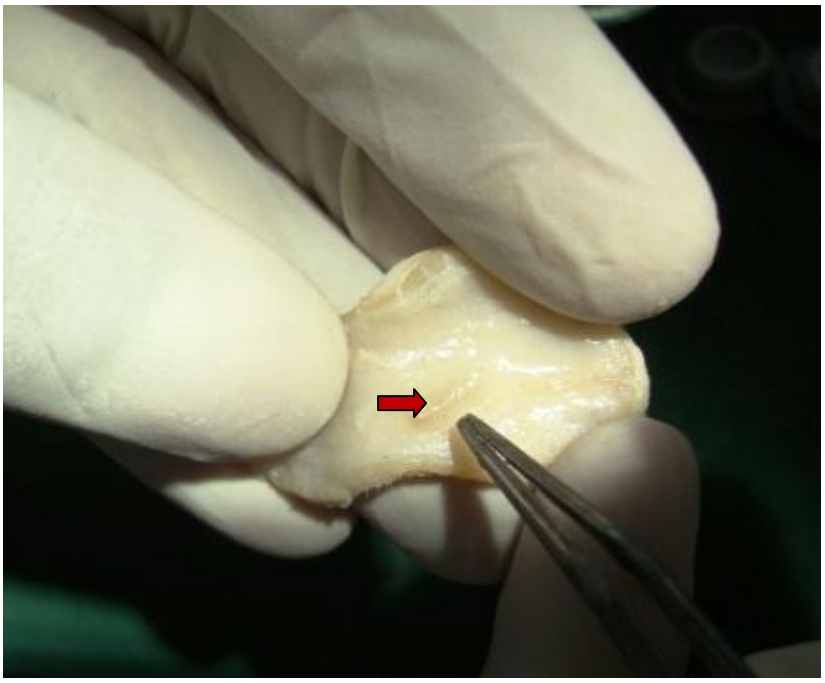


**Fig. 31 Leica Microtome**

**Fig. 32 Sectioning 4 µm thickness**

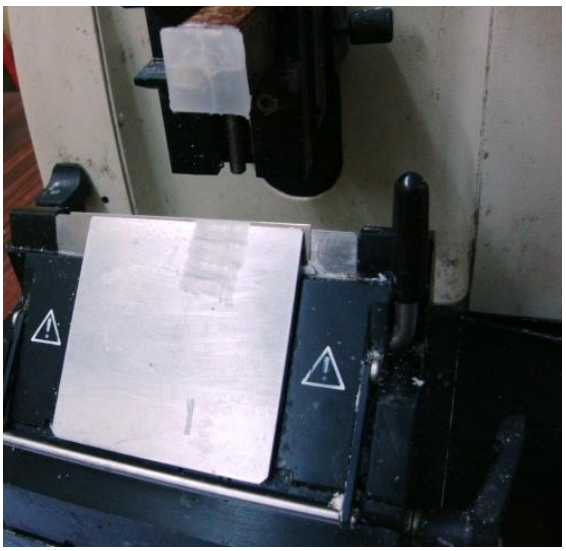


**Fig. 33 Leica semiautomatic stainer**

## TABLE –1

| GROUP CODE | MATERIAL | ANIMAL No. | SCORES OF INFLAMMATION | | |
|---|---|---|---|---|---|
| | | | 7 DAYS | 30 DAYS | 60 DAYS |
| GROUP I | CONTROL EMPTYTUBE | 1 | 0 | 0 | 0 |
| | | 2 | 0 | 1 | 0 |
| | | 3 | 2 | 0 | 1 |
| | | 4 | 2 | 1 | 0 |
| | | 5 | 1 | 1 | 1 |
| | | 6 | 1 | 0 | 0 |
| GROUP II | (MTA) | 1 | 2 | 2 | 1 |
| | | 2 | 2 | 1 | 2 |
| | | 3 | 2 | 2 | 2 |
| | | 4 | 3 | 1 | 1 |
| | | 5 | 2 | 2 | 1 |
| | | 6 | 1 | 2 | 1 |
| GROUP III | WPC+$Bi_2O_3$ | 1 | 3 | 1 | 1 |
| | | 2 | 1 | 2 | 2 |
| | | 3 | 2 | 1 | 1 |
| | | 4 | 2 | 2 | 1 |
| | | 5 | 2 | 2 | 2 |
| | | 6 | 2 | 2 | 2 |
| GROUP IV | WPC+$CHI_3$ | 1 | 2 | 1 | 2 |
| | | 2 | 1 | 2 | 1 |
| | | 3 | 2 | 1 | 2 |
| | | 4 | 2 | 2 | 1 |
| | | 5 | 2 | 2 | 1 |
| | | 6 | 2 | 1 | 1 |
| GROUP V | WPC+$ZrO_2$ | 1 | 2 | 2 | 2 |
| | | 2 | 3 | 2 | 2 |
| | | 3 | 2 | 1 | 1 |
| | | 4 | 3 | 2 | 2 |
| | | 5 | 2 | 2 | 1 |
| | | 6 | 2 | 2 | 2 |

# RESULT

No apparent adverse events were observed during the experimental periods. Macroscopic examination at the implant sites revealed that normal healing was satisfactory and without infection at all evaluation periods.

**Table 1** shows the inflammatory score obtained for the experimental groups. Each group was compared individually with others. Pearson Chi – Square test was used for the analysis. **Table – 2** to **Table -11** showed the individual comparison of inflammatory reactions of each group with the other.

In the 7 day experimental period, there was no statistical difference between groups. Moderate inflammation was seen in almost all groups. But most of the control (empty tube – Group I) showed few inflammatory cells.

In the 30 day experimental periods there was statistical difference between the Group I – control (empty tube) with the other groups. But there was no significant difference between Group II (MTA) with the other groups (Group III($WPC+Bi_2O_3$), Group IV($WPC+CHI_3$), Group V($WPC+ZrO_2$) and also between Group III, Group IV and Group V.

In 60 days, mild to moderate inflammation was present in Groups II, III, IV and V. No statistical difference was found between these groups. In Group I (control – empty tube) very mild inflammation with few inflammatory cells was present. In some specimens of Group I no inflammation was present. In the 60 day experimental period, there was statistical difference between Group I with other groups.

All groups in 7 days showed thin fibrous capsule formation. Fibrous capsule was increased in thickness in 30 days and it was more organised in 60 days.

**GROUP I (CONTRO**
**L) – EMPTY POLYETHYLENE TUBE**
**T – Empty tube, F – Fibrous capsule; Hematoxylin Eosin stain**

**Fig.34 After 7 days**
**Fibrous capsule was very thin**
**(X40 magnification)**

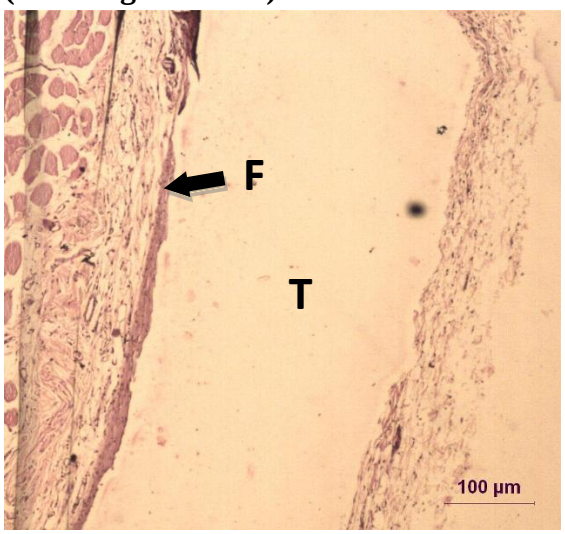



**Fig.35 After 30 days**
**(X40 Magnification)**

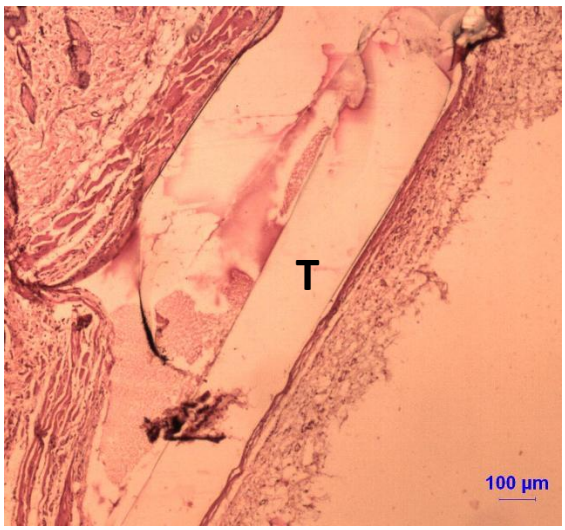



**Fig. 36 After 30 days.**
**(X 100 magnification)**

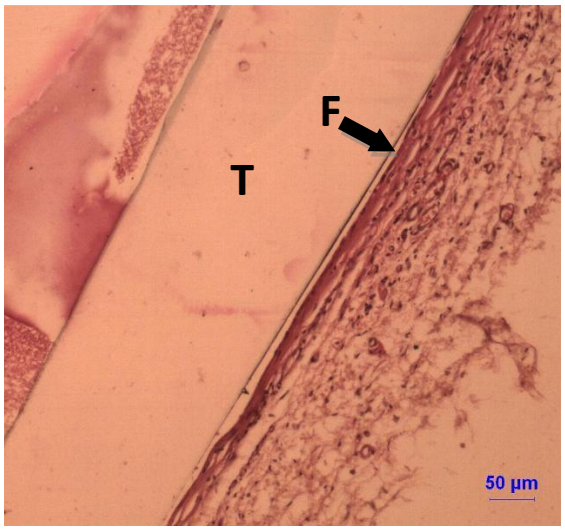
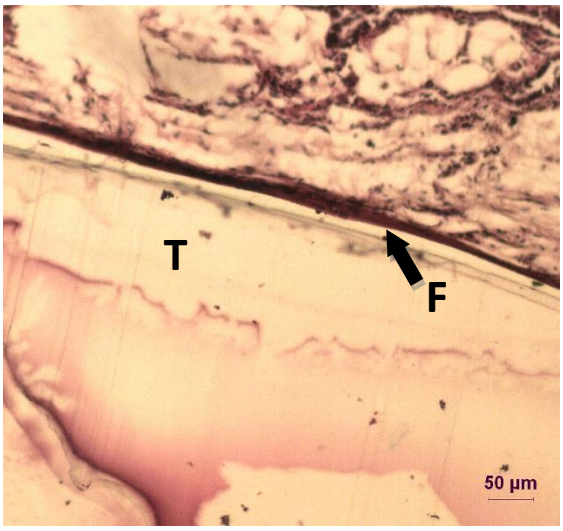



**Fig.37 After 60 days. (X100 magnification)**
**Fibrous capsule – thick & orgainsed**



**GROUP II – MTA**

**T – Tube loaded with MTA; F – Fibrous capsule;**
**M – Mononuclear infiltrate; I – Inflammatory cells. X – MTA material .**

**Fig. 38 After 7 days.**
**(X 100 magnification)**

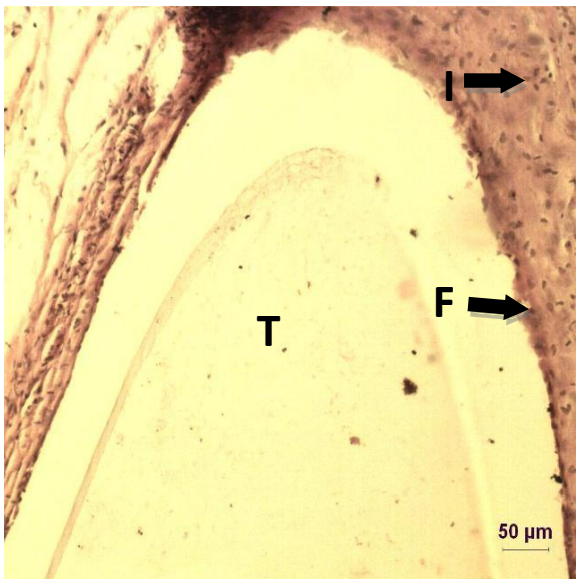



**Fig. 39 After 7 days showing severe mononuclear infiltration (X 200 magnification)**


**Fig. 40 After 30 days showing increased fibrous Capsule thickness & moderate inflammatory infiltration (X 100 magnification)**

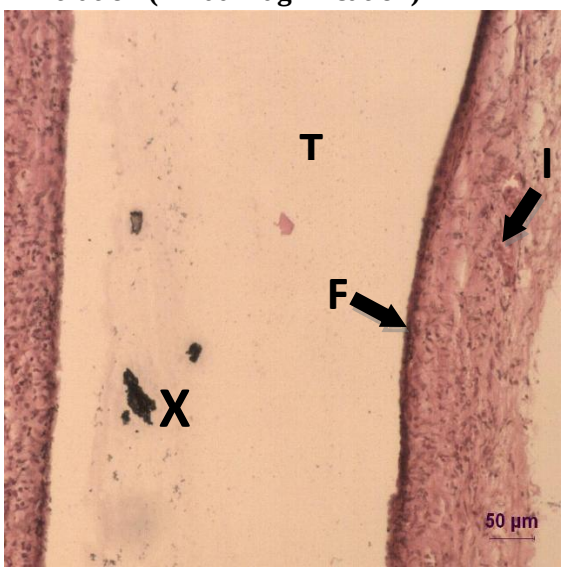
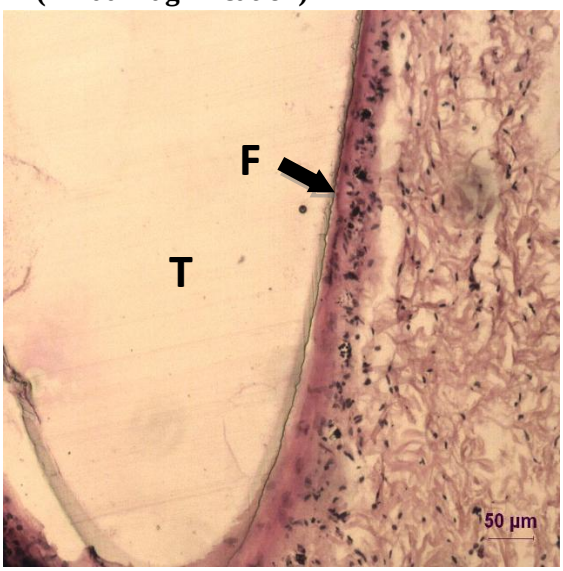



**Fig. 41 After 60 days showing organized & thick fibrous capsule.**
**(X 100 magnification)**



**GROUP III – WPC + $Bi_2O_3$**

**T – Tube; F – Fibrosis; I – Inflammatory cells; M – severe mononuclear infiltration; X – Group III material.**

**Fig. 42 After 7 days (X 100 magnification)**

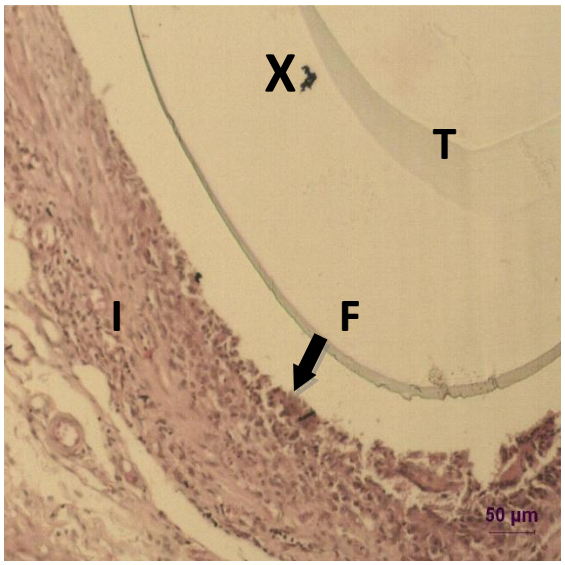



**Fig. 43 After 7 days showing severe mononuclear inflammatory infiltration. (X 200 magnification)**

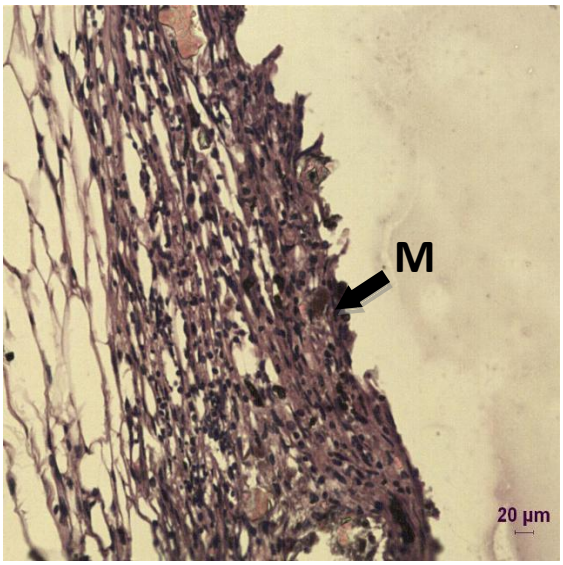



**Fig.44After 30 days showing moderate inflammatory reaction (X 40 magnification)**

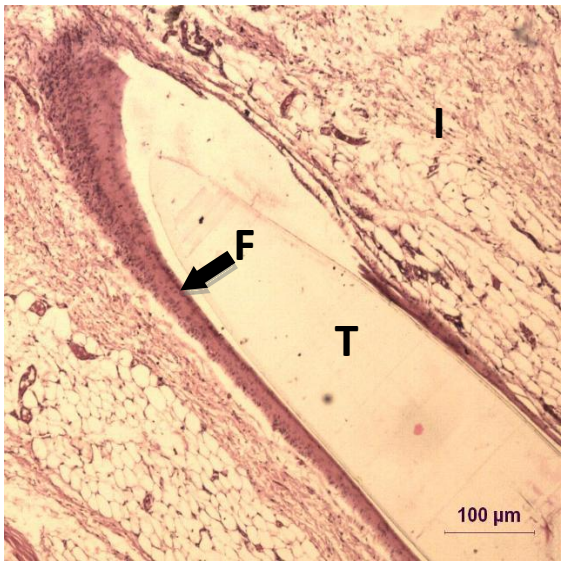
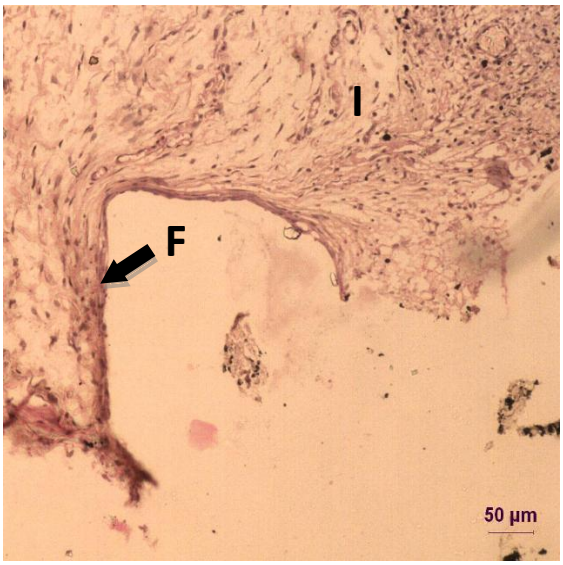



**Fig.45 After 60 days showing mild Inflammatory reaction with fibrous capsule formation (X 100 magnification)**



## GROUP IV – WPC + IODOFORM

**T – tube; F – Fibrous capsule; I – inflammatory cells; X – Group IV material**

**Fig. 46 After 7 days showing thin fibrous Capsule & moderate inflammation (X 100 magnification)**

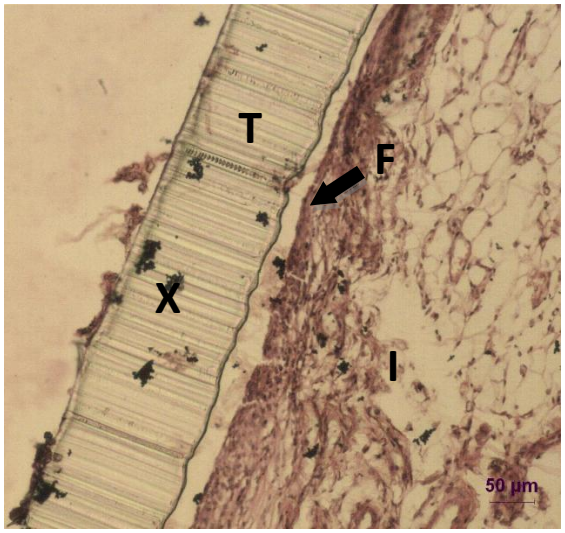



**Fig. 47 After 30 days showing increased thickness of fibrous capsule (X 100 magnifiation)**

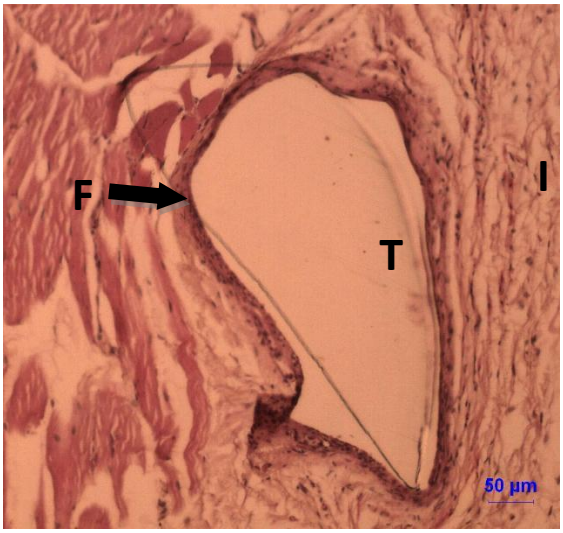



**Fig. 48 After 30 days (X 200 magnification)**

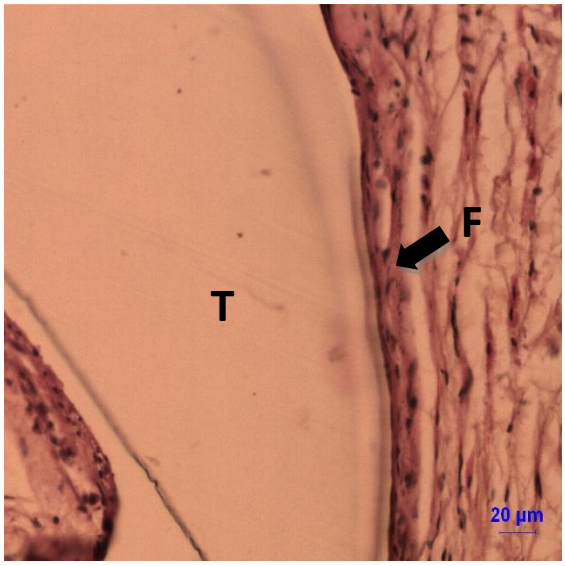
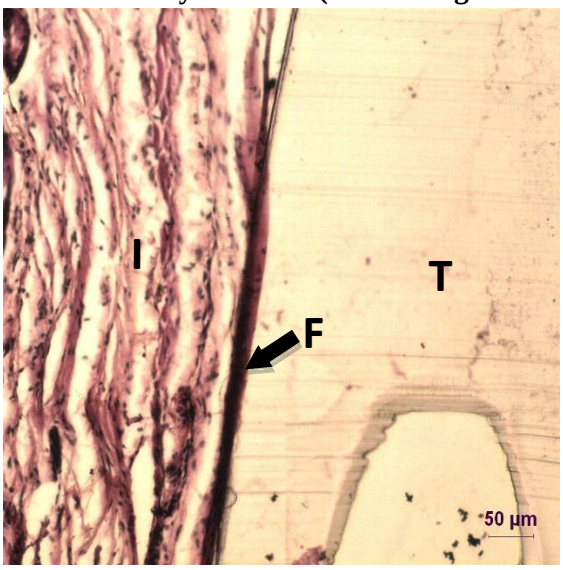



**Fig. 49 After 60 days showing increased thickness of fibrous capsule & mild Inflammatory reaction. ( X 100 magnification)**



## GROUP V – WPC + $ZrO_2$

**T – tube ; F – Fibrous Capsule; X – Group V material; M – Severe plasma cell infiltration; I – Chronic Inflammatory infiltration.**

**Fig. 50 After 7 days showing moderate Inflammatory infiltration with thin Fibrous capsule.(X 40 magnification)**

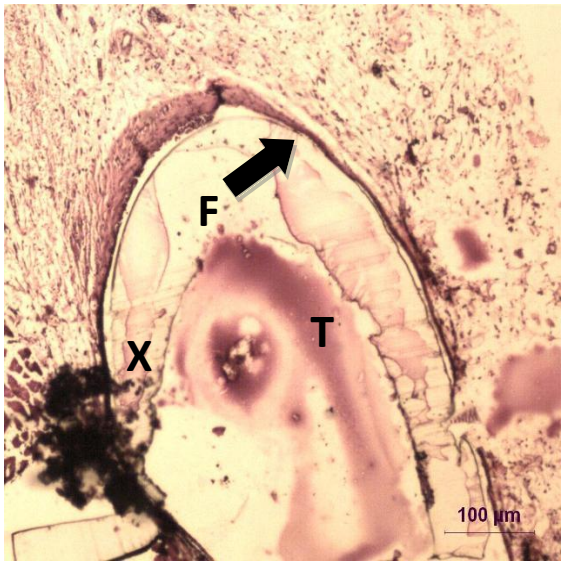



**Fig. 51 After 7 days showing severe plasma cell infiltration. (X 100 magnification)**

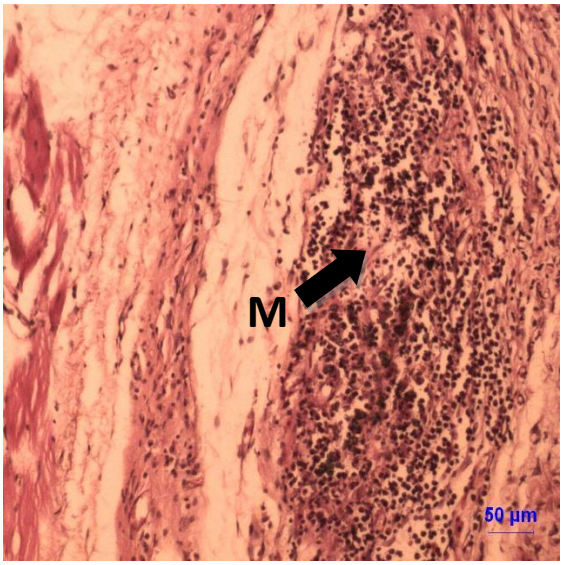



**Fig. 52 After 30 days showing fibrous Capsule & tube with material. (X 100magnification)**

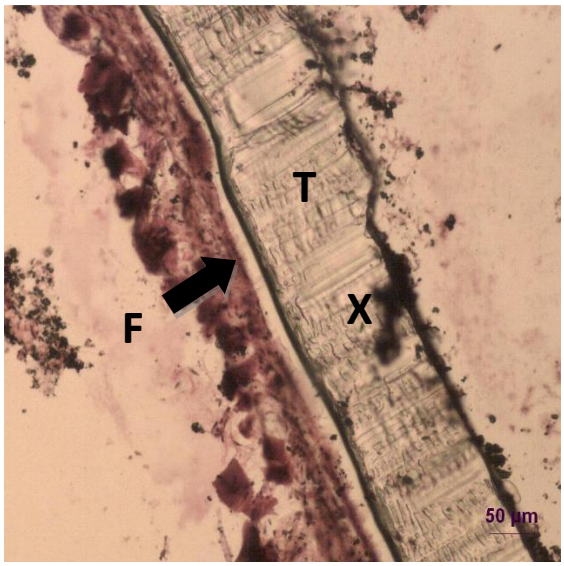
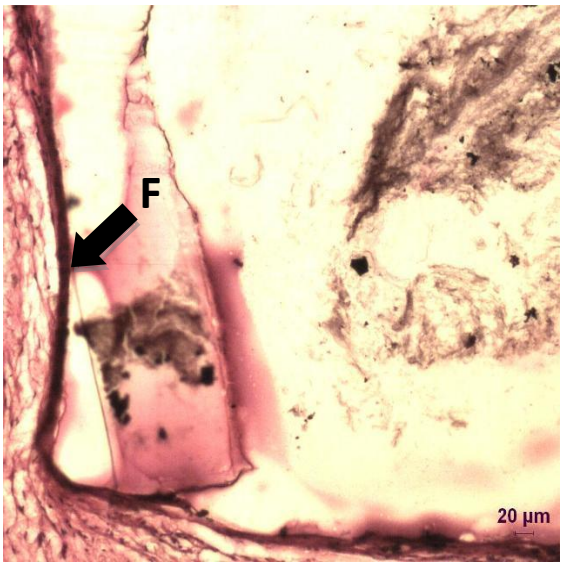



**Fig. 53 After 60 days showing organized & thick fibrous capsule with few inflammatory cells (X 200 magnification)**

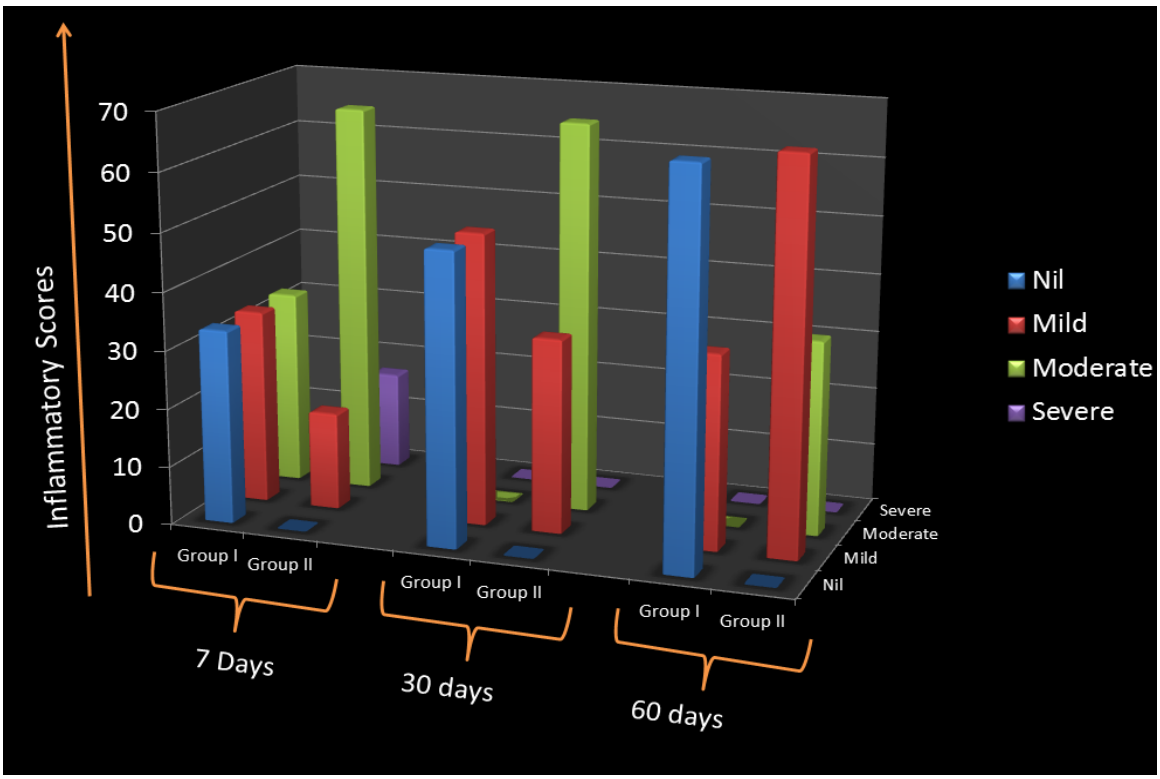


## Table – 2

**Comparison of inflammatory reaction between Group I and Group II in 7days, 30 days and 60 days**

| Day | Reactions | Group I | | Group II | | Chi-Value | P-Value |
|---|---|---|---|---|---|---|---|
| | | Counts | % | Counts | % | | |
| 7 | Nil | 2 | 33.3 | 0 | 0 | 4.000 | 0.261$ |
| | Mild | 2 | 33.3 | 1 | 16.7 | | |
| | Moderate | 2 | 33.3 | 4 | 66.7 | | |
| | Severe | 0 | 0 | 1 | 16.7 | | |
| 30 | Nil | 3 | 50 | 0 | 0 | 7.200 | 0.027* |
| | Mild | 3 | 50 | 2 | 33.3 | | |
| | Moderate | 0 | 0 | 4 | 66.7 | | |
| | Severe | 0 | 0 | 0 | 0 | | |
| 60 | Nil | 4 | 66.7 | 0 | 0 | 6.667 | 0.036* |
| | Mild | 2 | 33.3 | 4 | 66.7 | | |
| | Moderate | 0 | 0 | 2 | 33.3 | | |
| | Severe | 0 | 0 | 0 | 0 | | |

**Note: * - Significant; $ - Not Significant**

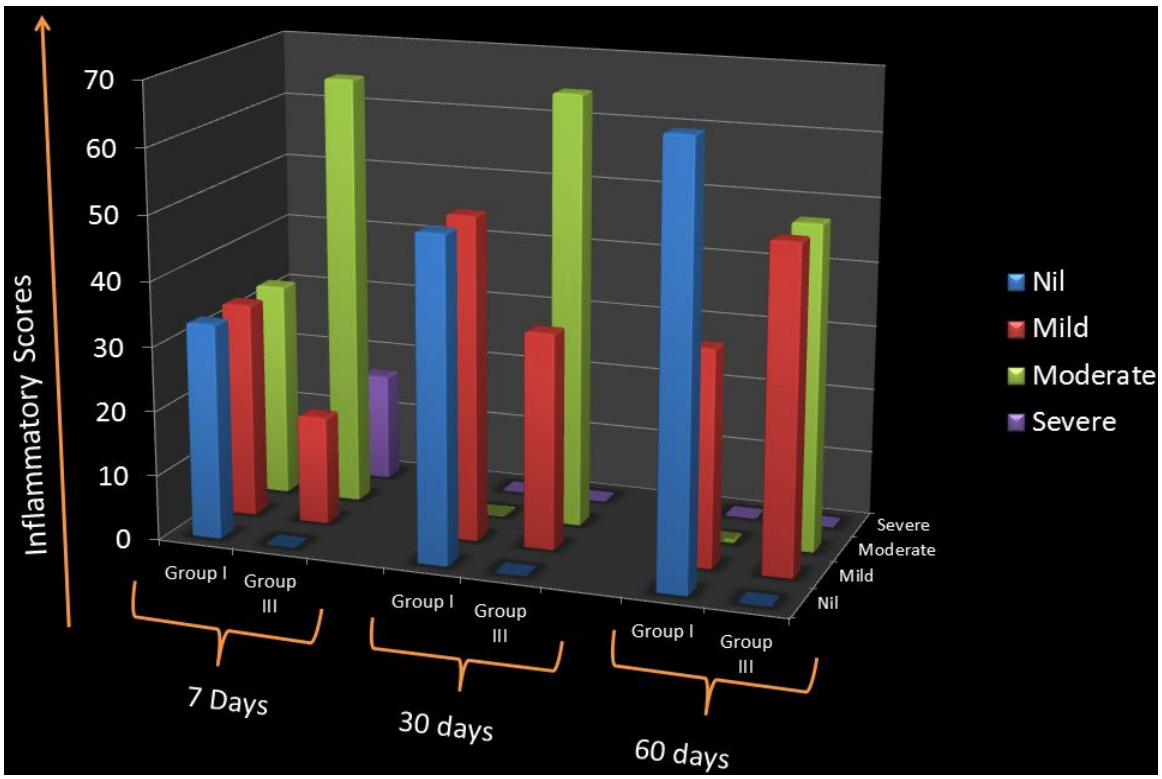


## Table - 3

**Comparison of inflammatory reaction between Group I and Group III in 7 days, 30 days and 60 days.**

| Days | Reactions | Group I | | Group III | | Chi-Value | P-Value |
|---|---|---|---|---|---|---|---|
| | | Counts | % | Counts | % | | |
| 7 | Nil | 2 | 33.3 | 0 | 0 | 4.000 | 0.261$ |
| | Mild | 2 | 33.3 | 1 | 16.7 | | |
| | Moderate | 2 | 33.3 | 4 | 66.7 | | |
| | Severe | 0 | 0 | 1 | 16.7 | | |
| 30 | Nil | 3 | 50 | 0 | 0 | 7.200 | 0.027* |
| | Mild | 3 | 50 | 2 | 33.3 | | |
| | Moderate | 0 | 0 | 4 | 66.7 | | |
| | Severe | 0 | 0 | 0 | 0 | | |
| 60 | Nil | 4 | 66.7 | 0 | 0 | 7.200 | 0.027* |
| | Mild | 2 | 33.3 | 3 | 50 | | |
| | Moderate | 0 | 0 | 3 | 50 | | |
| | Severe | 0 | 0 | 0 | 0 | | |

**Note: * - Significant; $ - Not Significant**

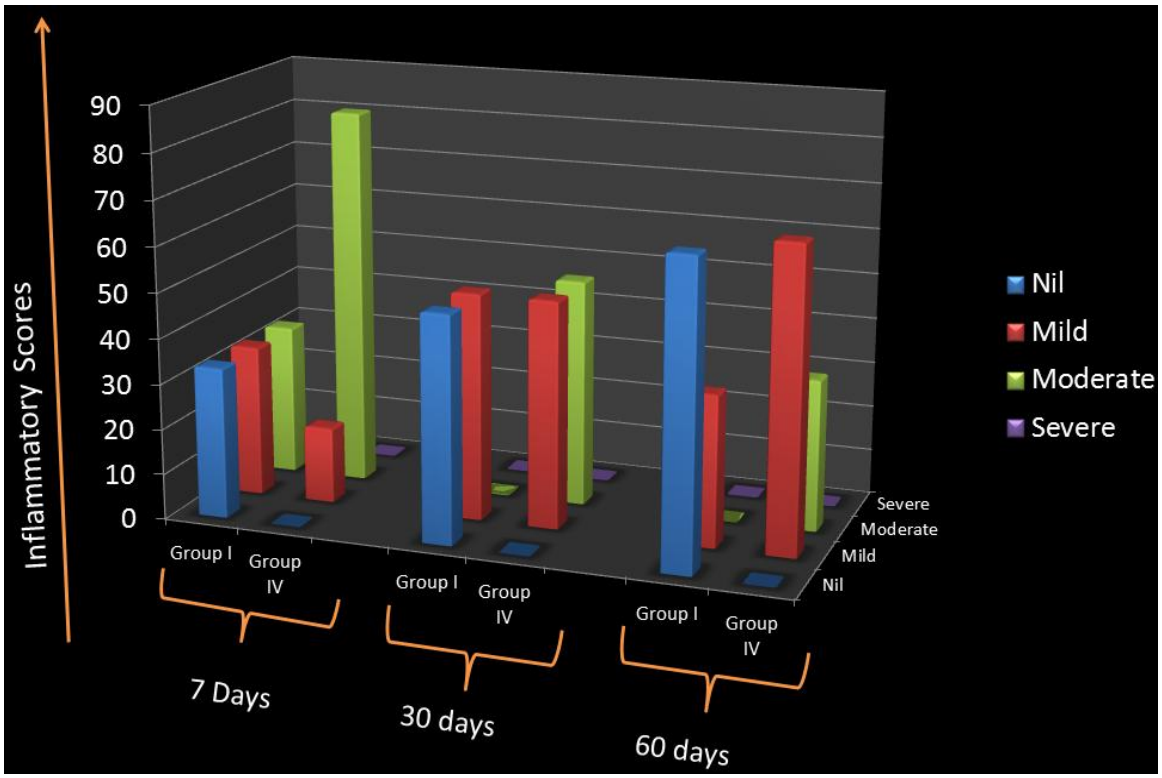


## Table 4

**Comparison of inflammatory reaction between Group I and Group IV in 7 days, 30 days and 60 days.**

| Days | Reactions | Group I | | Group IV | | Chi-Value | P-Value |
|---|---|---|---|---|---|---|---|
| | | Counts | % | Counts | % | | |
| 7 | Nil | 2 | 33.3 | 0 | 0 | 3.619 | 0.164$ |
| | Mild | 2 | 33.3 | 1 | 16.7 | | |
| | Moderate | 2 | 33.3 | 5 | 83.3 | | |
| | Severe | 0 | 0 | 0 | 0 | | |
| 30 | Nil | 3 | 50 | 0 | 0 | 6.000 | 0.048* |
| | Mild | 3 | 50 | 3 | 50 | | |
| | Moderate | 0 | 0 | 3 | 50 | | |
| | Severe | 0 | 0 | 0 | 0 | | |
| 60 | Nil | 4 | 66.7 | 0 | 0 | 6.667 | 0.036* |
| | Mild | 2 | 33.3 | 4 | 66.7 | | |
| | Moderate | 0 | 0 | 2 | 33.3 | | |
| | Severe | 0 | 0 | 0 | 0 | | |

**Note: * - Significant; $ - Not Significant**

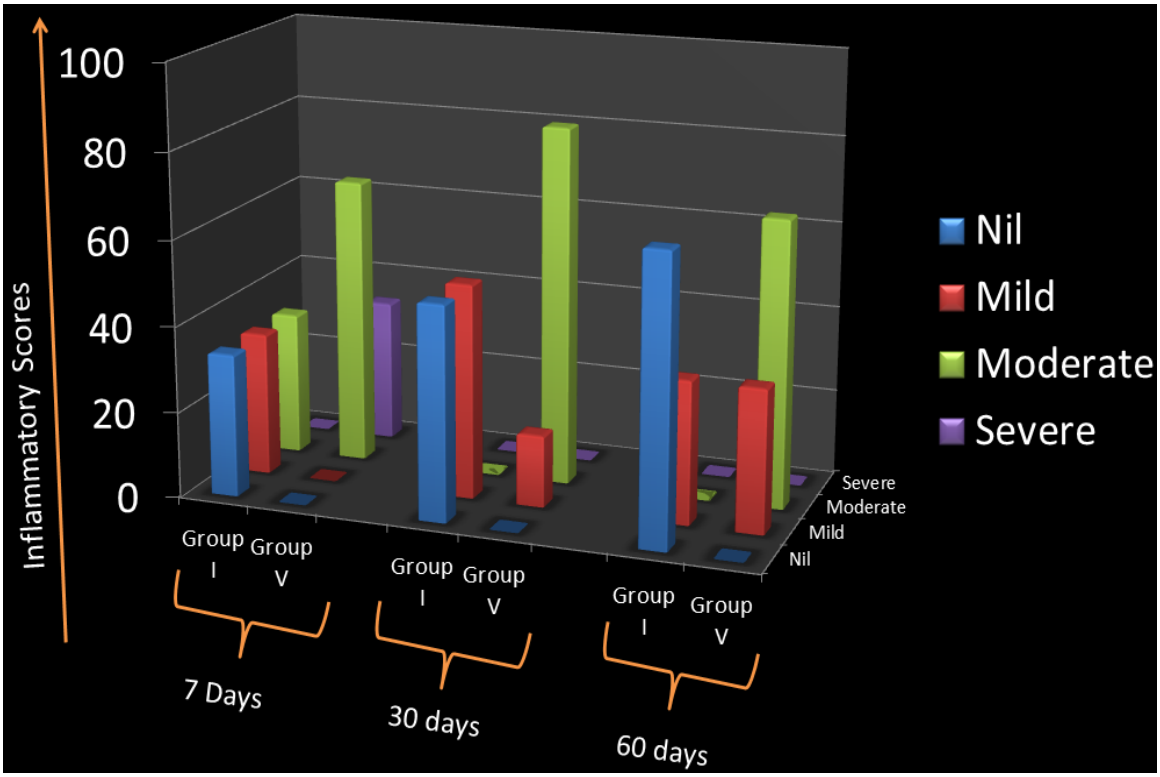


## Table 5

**Comparison of inflammatory reaction between Group I and Group V in 7 days, 30 days and 60 days.**

| Days | Reactions | Group I | | Group V | | Chi-Value | P-Value |
|---|---|---|---|---|---|---|---|
| | | Counts | % | Counts | % | | |
| 7 | Nil | 2 | 33.3 | 0 | 0 | 6.667 | 0.083$ |
| | Mild | 2 | 33.3 | 0 | 0 | | |
| | Moderate | 2 | 33.3 | 4 | 66.7 | | |
| | Severe | 0 | 0 | 2 | 33.3 | | |
| 30 | Nil | 3 | 50 | 0 | 0 | 9.000 | 0.011* |
| | Mild | 3 | 50 | 1 | 16.7 | | |
| | Moderate | 0 | 0 | 5 | 83.3 | | |
| | Severe | 0 | 0 | 0 | 0 | | |
| 60 | Nil | 4 | 66.7 | 0 | 0 | 8.000 | 0.018* |
| | Mild | 2 | 33.3 | 2 | 33.3 | | |
| | Moderate | 0 | 0 | 4 | 66.7 | | |
| | Severe | 0 | 0 | 0 | 0 | | |

**Note: * - Significant; $ - Not Significant**

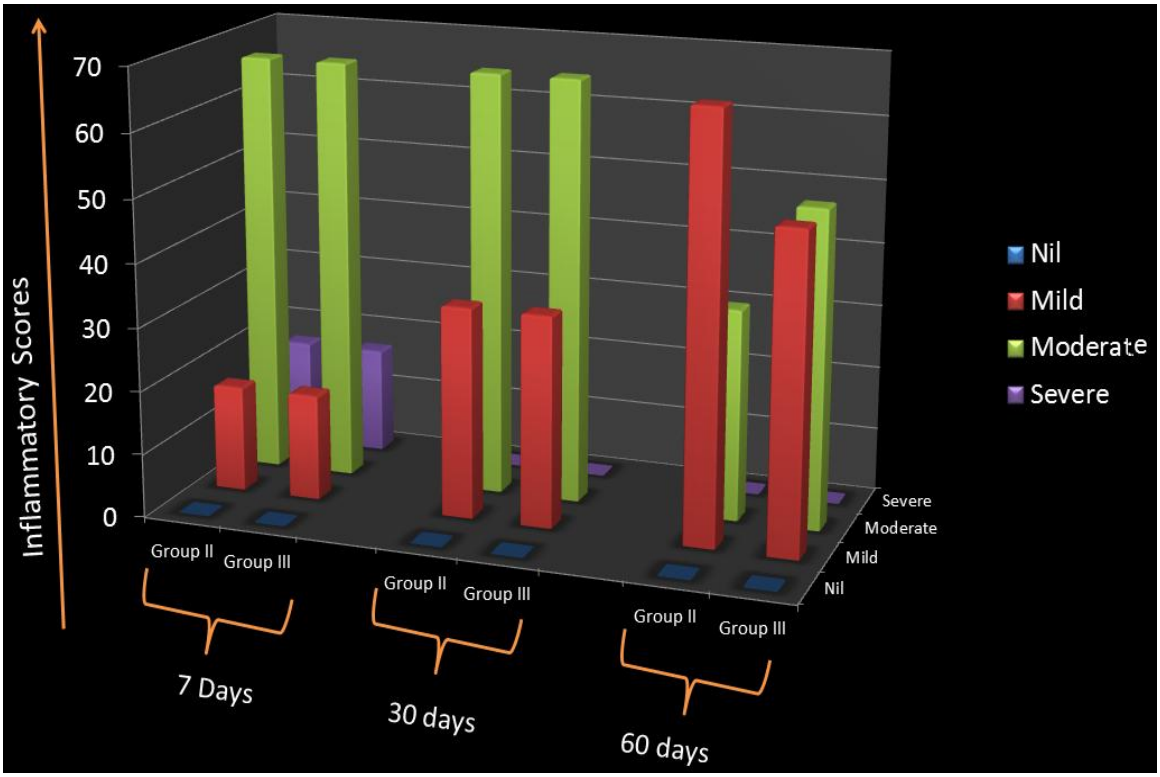


## Table 6

**Comparison of inflammatory reaction between Group II and Group III in 7 days, 30 days and 60 days.**

| Days | Reactions | Group II | | Group III | | Chi-Value | P-Value |
|---|---|---|---|---|---|---|---|
| | | Counts | % | Counts | % | | |
| 7 | Nil | 0 | 0 | 0 | 0 | .000 | 1.000$ |
| | Mild | 1 | 16.7 | 1 | 16.7 | | |
| | Moderate | 4 | 66.7 | 4 | 66.7 | | |
| | Severe | 1 | 16.7 | 1 | 16.7 | | |
| 30 | Nil | 0 | 0 | 0 | 0 | .000 | 1.000$ |
| | Mild | 2 | 33.3 | 2 | 33.3 | | |
| | Moderate | 4 | 66.7 | 4 | 66.7 | | |
| | Severe | 0 | 0 | 0 | 0 | | |
| 60 | Nil | 0 | 0 | 0 | 0 | 0.343 | 0.558$ |
| | Mild | 4 | 66.7 | 3 | 50 | | |
| | Moderate | 2 | 33.3 | 3 | 50 | | |
| | Severe | 0 | 0 | 0 | 0 | | |

**Note: * - Significant; $ - Not Significant**

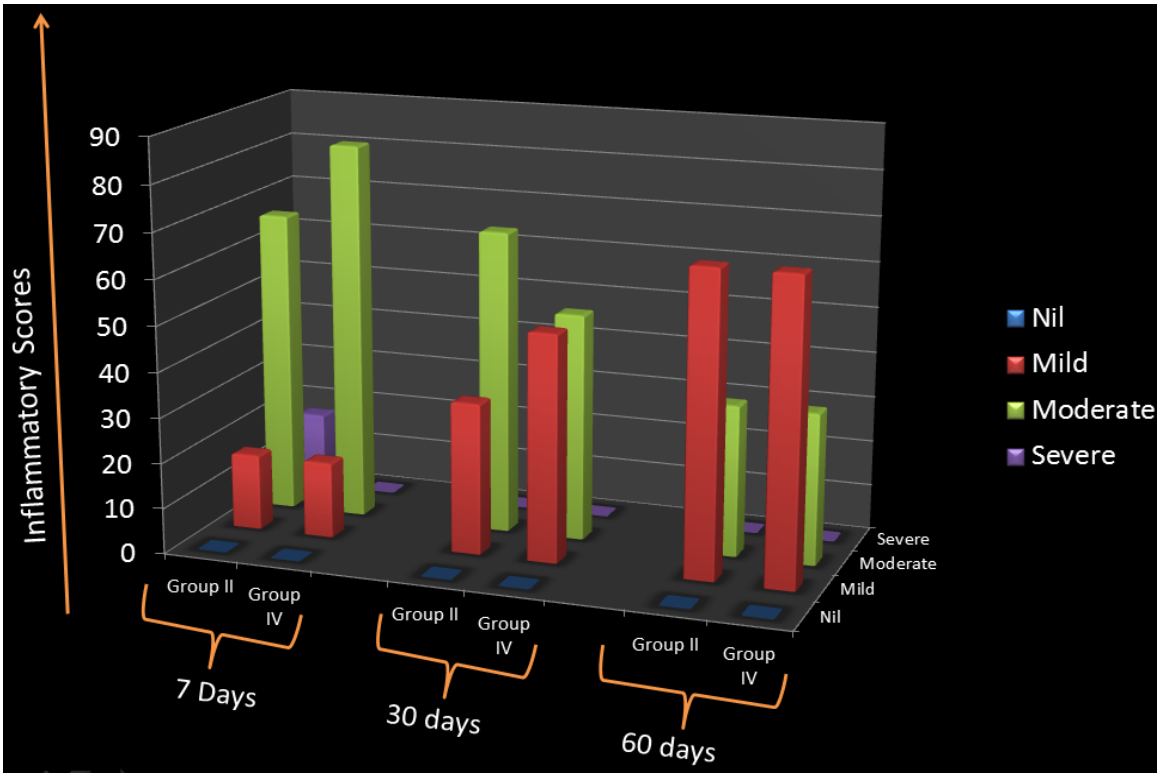


## Table 7

**Comparison of inflammatory reaction between Group II and Group IV in 7 days, 30 days and 60 days.**

| Days | Reactions | Group II | | Group IV | | Chi-Value | P-Value |
|---|---|---|---|---|---|---|---|
| | | Counts | % | Counts | % | | |
| 7 | Nil | 0 | 0 | 0 | 0 | 1.111 | 0.574$ |
| | Mild | 1 | 16.7 | 1 | 16.7 | | |
| | Moderate | 4 | 66.7 | 5 | 83.3 | | |
| | Severe | 1 | 16.7 | 0 | 0 | | |
| 30 | Nil | 0 | 0 | 0 | 0 | 0.343 | 0.558$ |
| | Mild | 2 | 33.3 | 3 | 50 | | |
| | Moderate | 4 | 66.7 | 3 | 50 | | |
| | Severe | 0 | 0 | 0 | 0 | | |
| 60 | Nil | 0 | 0 | 0 | 0 | 0.000 | 1.000$ |
| | Mild | 4 | 66.7 | 4 | 66.7 | | |
| | Moderate | 2 | 33.3 | 2 | 33.3 | | |
| | Severe | 0 | 0 | 0 | 0 | | |

**Note: * - Significant; $ - Not Significant**

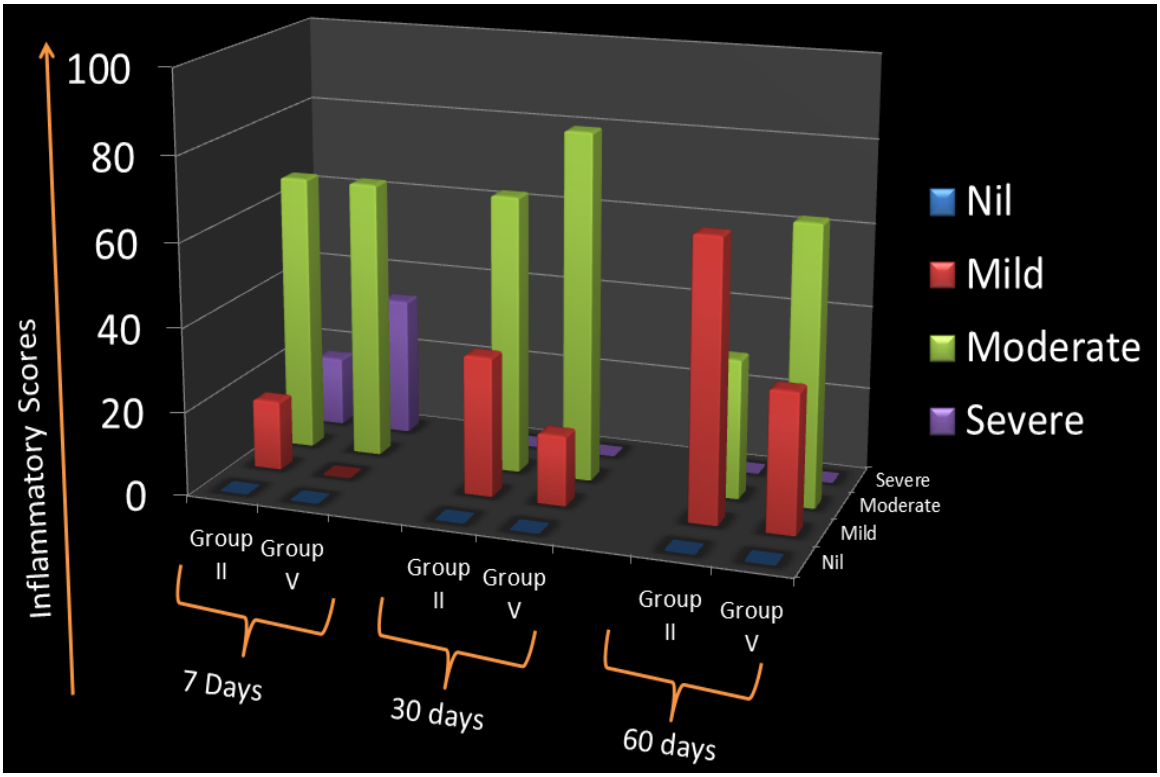


## Table 8

**Comparison of inflammatory reaction between Group II and Group V in 7 days, 30 days and 60 days.**

| Days | Reactions | Group II | | Group V | | Chi-Value | P-Value |
|---|---|---|---|---|---|---|---|
| | | Counts | % | Counts | % | | |
| 7 | Nil | 0 | 0 | 0 | 0 | 1.333 | 0.513$ |
| | Mild | 1 | 16.7 | 0 | 0 | | |
| | Moderate | 4 | 66.7 | 4 | 66.7 | | |
| | Severe | 1 | 16.7 | 2 | 33.3 | | |
| 30 | Nil | 0 | 0 | 0 | 0 | 0.444 | 0.505$ |
| | Mild | 2 | 33.3 | 1 | 16.7 | | |
| | Moderate | 4 | 66.7 | 5 | 83.3 | | |
| | Severe | 0 | 0 | 0 | 0 | | |
| 60 | Nil | 0 | 0 | 0 | 0 | 1.333 | 0.248$ |
| | Mild | 4 | 66.7 | 2 | 33.3 | | |
| | Moderate | 2 | 33.3 | 4 | 66.7 | | |
| | Severe | 0 | 0 | 0 | 0 | | |

**Note: * - Significant; $ - Not Significant**



## Table 9

**Comparison of inflammatory reaction between Group III and Group IV in 7 days, 30 days and 60 days.**

| Days | Reactions | Group III | | Group IV | | Chi-Value | P-Value |
|---|---|---|---|---|---|---|---|
| | | Counts | % | Counts | % | | |
| 7 | Nil | 0 | 0 | 0 | 0 | 1.111 | 0.574$ |
| | Mild | 1 | 16.7 | 1 | 16.7 | | |
| | Moderate | 4 | 66.7 | 5 | 83.3 | | |
| | Severe | 1 | 16.7 | 0 | 0 | | |
| 30 | Nil | 0 | 0 | 0 | 0 | 0.343 | 0.558$ |
| | Mild | 2 | 33.3 | 3 | 50 | | |
| | Moderate | 4 | 66.7 | 3 | 50 | | |
| | Severe | 0 | 0 | 0 | 0 | | |
| 60 | Nil | 0 | 0 | 0 | 0 | 0.343 | 0.558$ |
| | Mild | 3 | 50 | 4 | 66.7 | | |
| | Moderate | 3 | 50 | 2 | 33.3 | | |
| | Severe | 0 | 0 | 0 | 0 | | |

**Note: * - Significant; $ - Not Significant**

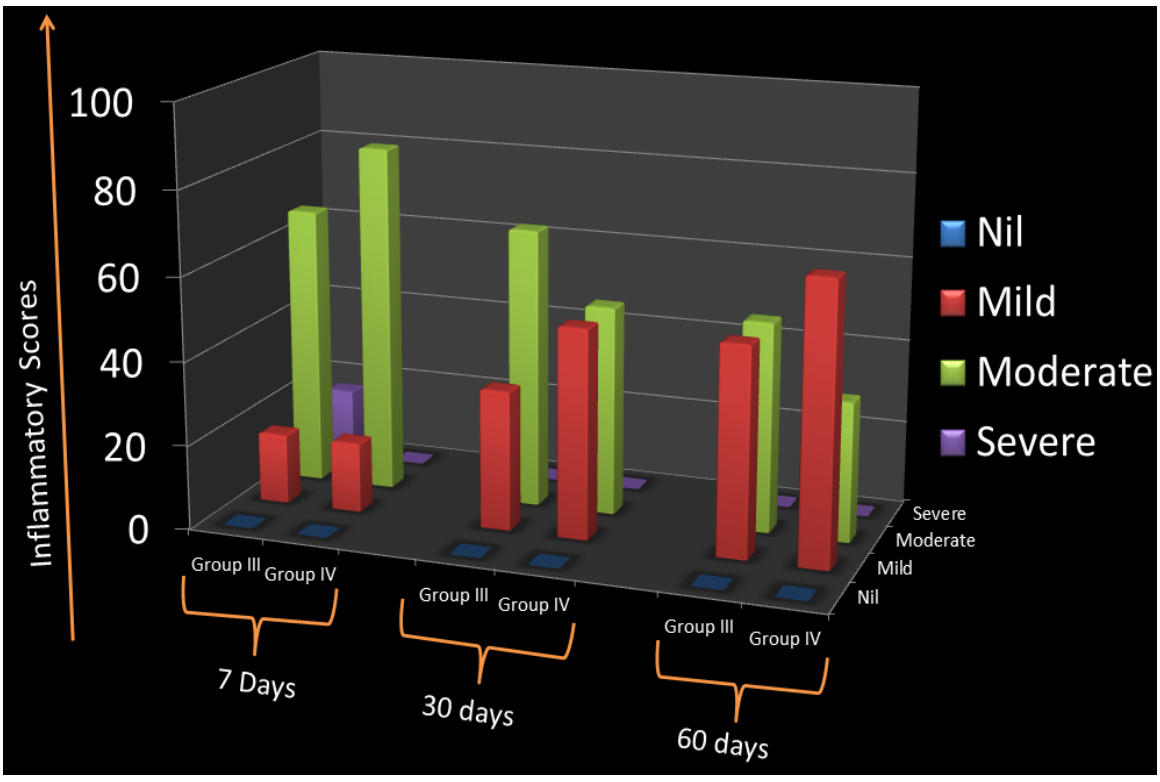
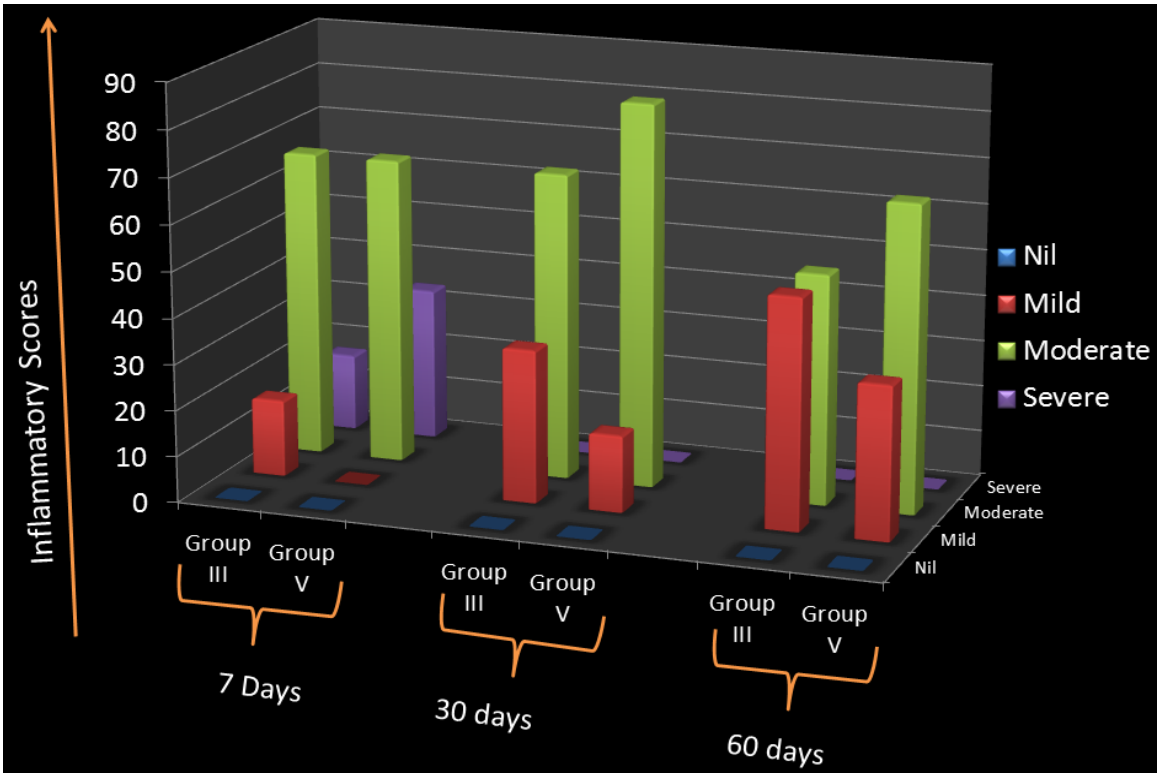


**Table 10**

**Comparison of inflammatory reaction between Group III and Group V in 7 days, 30 days and 60 days.**

| Days | Reactions | Group III | | Group V | | Chi-Value | P-Value |
|---|---|---|---|---|---|---|---|
| | | Counts | % | Counts | % | | |
| 7 | Nil | 0 | 0 | 0 | 0 | 1.333 | 0.513$ |
| | Mild | 1 | 16.7 | 0 | 0 | | |
| | Moderate | 4 | 66.7 | 4 | 66.7 | | |
| | Severe | 1 | 16.7 | 2 | 33.3 | | |
| 30 | Nil | 0 | 0 | 0 | 0 | 0.444 | 0.505$ |
| | Mild | 2 | 33.3 | 1 | 16.7 | | |
| | Moderate | 4 | 66.7 | 5 | 83.3 | | |
| | Severe | 0 | 0 | 0 | 0 | | |
| 60 | Nil | 0 | 0 | 0 | 0 | 0.343 | 0.558$ |
| | Mild | 3 | 50 | 2 | 33.3 | | |
| | Moderate | 3 | 50 | 4 | 66.7 | | |
| | Severe | 0 | 0 | 0 | 0 | | |

**Note: * - Significant; $ - Not Significant**

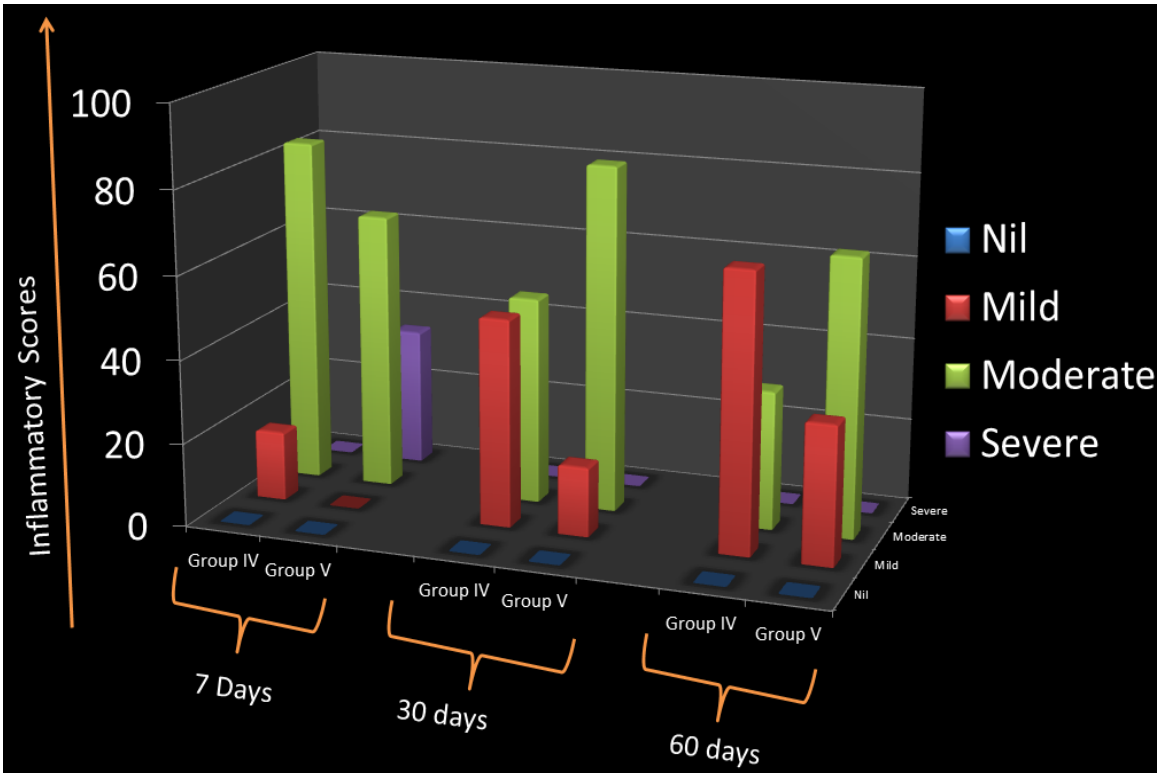


## Table 11

**Comparison of inflammatory reaction between Group IV and Group V in 7 days, 30 days and 60 days.**

| Days | Reactions | Group IV | | Group V | | Chi-Value | P-Value |
|---|---|---|---|---|---|---|---|
| | | Counts | % | Counts | % | | |
| 7 | Nil | 0 | 0 | 0 | 0 | 3.111 | 0.211$ |
| | Mild | 1 | 16.7 | 0 | 0 | | |
| | Moderate | 5 | 83.3 | 4 | 66.7 | | |
| | Severe | 0 | 0 | 2 | 33.3 | | |
| 30 | Nil | 0 | 0 | 0 | 0 | 1.500 | 0.221$ |
| | Mild | 3 | 50 | 1 | 16.7 | | |
| | Moderate | 3 | 50 | 5 | 83.3 | | |
| | Severe | 0 | 0 | 0 | 0 | | |
| 60 | Nil | 0 | 0 | 0 | 0 | 1.333 | 0.248$ |
| | Mild | 4 | 66.7 | 2 | 33.3 | | |
| | Moderate | 2 | 33.3 | 4 | 66.7 | | |
| | Severe | 0 | 0 | 0 | 0 | | |

**Note: * - Significant; $ - Not Significant**



## DISCUSSION:

The growing technological evolution and continuous introduction of endodontic materials for different applications make the evaluation of the biological properties of these new products a mandatory condition. Biocompatibility is one of the most important properties of endodontic materials because it will be in contact permanently with living tissues in the periapical region. Materials used in root end filling, furcal perforation and as apical barrier must have their biocompatibility characteristics investigated. Currently there are three recommended tests for biologic evaluation and acceptance of endodontic materials, 1) a general toxicity profile for materials; 2) a secondary tests, which evaluates its local toxicity; 3) a usage tests in vivo which precede clinical trials in animals and then humans[18].

The secondary or local toxicity tests were designed to produce evidence of subacute toxicity after longer periods in soft or hard tissues, essentially for screening purposes[33]. It is clear that one cannot extrapolate data to humans based on the results of experiments on animals. However, the introduction of different materials in the subcutaneous tissues of small laboratory animals are widely regarded as valid procedures for the study of their biological properties[47].

Other than subcutaneous tissue, implantation into bone[36,45] or the test materials were directly applied into the subcutaneous tissues[62] were suggested methods for secondary toxicity tests. The implantation of the materials in tubes

was advocated in many studies[47,48,54,31,11,63]. The materials were placed into tubes in order to simulate the clinical situation. Also the use of tubes is more suitable for unset or soft materials. When compared with the direct application of the material, the subcutaneous implantation in tubes would help to provide stabilization of the material placement and to achieve the standardization of the material – tissue interface[61].

In this study we decided to test the materials using subcutaneous tissue implantation procedure because it was a simple, fast and economical method.

The animal used in this study was wistor albino rats, 18 in number weighing 200±20gms. Wistor rats are an outbred strains of albino rats belonging to the species Rattus norvegicus. This strain was developed at the Wistar Institute in 1906 for use in biological and medical research, and is notably the first rat strain developed to serve as a model organism. The Wistor rat is currently one of the most popular rat strain used for laboratory research.

Different kind of tubes had been used, dentin tubes[25] , polyethylene tubes[31], polystyrene tubes[61], polypropylene tubes[31] and silicon tubes[64] in various studies. The implantation test was refined in the 1960's when Torneck showed that subsequent to the implantation of polyethylene tubes, fibrous tissue repair occurred with no lasting inflammation[12].Empty polyethylene tubes that were sterile produce condition that were more favourable for repair. Polyethylene tubes were considered a suitable medium to carry the test materials[13]. In this

study we used polyethylene tubes of inner diameter 1.2 mm and length 5mm to carry the test materials.

Portland cement is most widely employed material in construction. Better characterized by Joseph Asdpin in 1824, because it resembled the colour of stones of the Isle of Portland of south of England, which was the material used in construction at that time.

Portland cement manufactured by a clinkering process or partial fusion of raw materials. This process includes lime stone decarbonisation at 400$^{o}$ to 600$^{o}$C; formation of dicalcium silicate, tricalcium silicate and tricalcium aluminoferrite between 800$^{o}$ and 1200 $^{o}$C; and production of tricalcium silicate at 1400$^{o}$C by the reaction of dicalcium silicate with free lime. Additives may be included and depending on the type of additives, Portland cement is classified into different types[56].

White Portland cement (WPC) differs from ordinary Portland cement in its lower iron content. The lighter colour of WPC is because of the reduction in the ferrite phase. During its production WPC, the ferrite component is usually reduced by producing the cement clinker under reducing conditions and by rapid quenching. WPC also has lower compressive strength compared with ordinary Portland cement and is used commercially in civil engineering works as a repair material and in architecture because of its aesthetic value[8].

Type I Portland cement was used as the main componenet of MTA. According to its manufacturer, Pro Root MTA has its composition as 75% Portland cement, 20% Bismuth oxide and 5% dehydrated calcium sulphate. Pro Root MTA was approved for use by the Food and Drug Administration (FDA) in 1998 after extensive in vitro and in vivo tests demonstrated its biocompatibility.

Sealing ability of MTA as root end fillings found to be superior than amalgam and super EBA and IRM[37,55].MTA exhibits acceptable in vivo biologic performance when used for root-end fillings, perforation repairs, pulp capping and pulpotomy, and apexification treatment[58,59]. MTA induces biomineralization of cementoblasts[24] and stimulate mineralization[30].

In this study we used Pro Root MTA, tooth colored formula (Dentsply,USA) and white Portland cement – Birla white (Grasim Ind ltd. Aditya Birla group).

Similar chemical elements were found in Portland cement and Pro Root MTA and there was a small percentile variation among them. In spite of the chemical similarity, a difference in the texture and size were observed. Pro Root MTA presented the highest percentage of bismuth oxide (9.2% on average). Except for bismuth oxide, Portland cement and MTA cements presented similar chemical formulation[40]. The X-ray diffraction results indicated that tricalcium silicate, tricalcium aluminate, dicalcium silicate and tetracalcium aluminoferrite were the major constitutents of WPC, Portland cement and Pro Root MTA[26].

MTA and Portland cement had similar hydration mechanism[9]. MTA based materials and Portland cement contain calcium oxide, which when mixed with water, forms calcium hydroxide, inducing an increase of pH by dissociation of calcium and hydroxide ion, as demonstrated by Durate et al[19].

The antimicrobial activity of MTA seems to be associated with elevated pH. Torabinejad observed an initial pH of 10.2 for MTA, rising to 12.5 in 3hr. It is known that pH levels in the order of 12.0 can inhibit most microorganisms including resistant bacteria such as E. faecalis.[35] MTA and Portland cement had similar antimicrobial activity, suggesting that the addition of a radiopacifying agent to Portland cement during the manufacture of MTA – based materials does not hinder its antimicrobial action[41]. MTA and Portland cement show comparative biocompatibility when evaluated in vitro and in vivo[31].

The presence of heavy metals especially arsenic in MTA and Portland cement was of a major concern in their use in medicine. According to ISO 9917-1 standard, water – based cements: Powder/liquid acid base cements (2003), a material to be used in dental procedures should contain no more arsenic than 2 mg/kg of cement. The amount of arsenic in Pro Root MTA is 5.25 mg/kg, in white Portland cement 0.5 mg/kg and in gray Portland cement 34.27 mg/kg. It must be emphasized that MTA is used in very small amounts, less than 1 gm in clinical endodontic procedure. Thus 34.27 mg of arsenic per

kg of ordinary Portland cement correspond to 34.27µg of arsenic per gm, which is well below the lethal dose of 2 mg/kg body weight[7].

Similarly the release of arsenic in Portland cement and MTA, the highest values were observed for Portland cement with values of 0.007 ppm after 3 hr and 0.006 ppm after 168 h and MTA with values 0.002 ppm after 3 hr and 168 hr. All of these values were well below the toxic levels[38].

Lack of acceptable radiopacity was a major concern when considering the use of Portland cement as a substitute to Pro Root MTA, because visual differentiation of the material from the surrounding tissues was needed in the radiograph. Radiopacifying agents must be added to Portland cement in order to solve this problem.

The ISO 6876/2001 standard establishes that root canal sealer should be atleast as radiopaque as 3 mm Al equivalent. According to ANSI American National Standard Institute and American Dental Association (ADA) Sp. No.57, endodontic filling material should present a difference in radiopacity equivalent to atleast 2mm Al in comparison to bone or dentin.

Radiopacity of Pro Root MTA was found to be 6.74 mmAl eq and that of WPC was 0.95 mmAl eq.[29]

Marco Antonio evaluated the radiopacity of Portland cement associated with various radiopacifying agents: Bismuth oxide, Zinc oxide, Iodoform, Calcium tungstate, Lead oxide, Bismuth carbonate, Barium sulphate and Zirconium oxide. A ratio of 20%wt radiopacifier and 80%wt Portland cement

was used. Bismuth oxide, Iodoform and Zirconium dioxide when added with Portland cement had radiopacity values 5.93mmAl, 4.24mmAl and 3.41mmAl respectively. All of the values are above that recommended by ADA/ANSI[39].

Carlos et al proved that atleast 15% Bismuth oxide must be added to WPC to give it sufficient radiopacity for it to be used as an endodontic material[10]. In MTA, 20% Bismuth oxide is the radiopacifier used.

In this study we used Bismuth oxide, Iodoform and Zirconium dioxide as the radiopacifiers to be mixed with the Portland cement. All the radiopacifying agents, bismuth oxide, iodoform and zirconium dioxide were mixed with white Portland cement in the ratio of 4:1 (i.e., 80wt% WPC + 20wt% radiopacifying agents).

Bismuth oxide was selected as one of the radiopacifier, so that we can compare the tissue reaction of Portland cement + Bismuth oxide to that of MTA, which also contains bismuth oxide as the radiopacifier.

Coomarswamy et al in his study discussed the effects of bismuth oxide on MTA. He proved that the addition of bismuth oxide decreased the mechanical stability by introducing flaws and increased porosity by leaving more unreacted water within the Portland cement based (MTA – like) materials. This affects the longevity of the material[15].But Saliba et al proved that addition of bismuth oxide did not seem to affect the compressive strength and other physical properties of Portland cement[49]. Since there were different school of thought there was a need to search for an alternative radiopacifying agent.

Hence we used Iodoform and Zirconium dioxide as radiopacifying agents. The choice of Iodoform as a radiopaque agent to be added to the WPC is due to its good radiopacity, prompt availability to the clinician.

Portland cement because of its manufacture involving temperature around 1500$^{o}$C and its alkalinity were mostly sterile. But contamination could occur during packaging, shipping and storage. In this study, WPC were sterilized by dry heat sterilization. James et al proved that dry heat sterilization as an effective way to render Portland cement sterile[29].

7 days after the surgical procedure Group I control (empty tube) showed mild inflammatory reaction with few inflammatory cells. The reaction to the empty polyethylene tube in this study was similar to those obtained in other studies[12,61,42]. Empty polyethylene tubes that were sterile and clean produce conditions that were more favourable for repair[13]. This was similar to other studies that reported that there were some inflammation around the empty tubes until the end of the second week and this inflammatory infiltration subsided after 4$^{th}$ week[12,61]. This reaction was the result of the trauma produced during the placement of the tubes[42]. The present work also showed that an acute inflammatory reaction at 7 days was a result of the surgical intervention.

At 7 days after surgical procedure, all the test materials Group II (MTA), Group III (WPC+$Bi_2O_3$), Group IV (WPC+$CHI_3$) and Group V (WPC+$ZrO_2$) showed moderate inflammatory response. One of the specimen in Group II and

Group III and two of the specimens in Group V after 7 days had severe mononuclear inflammatory infiltration. But no severe inflammatory response was seen in Group IV (Iodoform), only moderate inflammatory response was seen. The incorporation of 20wt% iodoform as a radiopaque agent to the WPC may be the reason for this, because of iodoform's anti-inflammatory property.

30 days after surgical procedure, no severe inflammatory response were observed in all the test materials. Mild to moderate inflammatory response were obtained. Similar results were observed in the previous studies[11,47,48]

60 days after surgical procedure, the inflammation were subsided in all the groups when comparing the 7 day response. In Group I (control – empty tube) mild inflammation with very few inflammatory cells were seen. The inflammation present in the Group I (empty tube – control) at 7 days subsides gradually in 30 days and there was almost no inflammation at 60 days. In both 30 and 60 days inflammatory response in Group I showed significant difference from all the test materials. No significant difference was found 7 days after surgical procedure. This was similar to results obtained in other studies[11,48].

After 60 days all the groups II, III, IV and V showed mild to moderate inflammatory response, even though the initial inflammatory response at 7 day was reduced. There was no significant difference between these groups.

In this study, Group II (MTA) and Group III (WPC + $Bi_2O_3$) had similar inflammatory response at all the experimental periods 7, 30 and 60 days. A previous study by Tauby et al compared the tissue reaction of MTA and WPC +

$Bi_2O_3$ and observed no difference between MTA and WPC and the addition of $Bi_2O_3$ did not interfere with the biocompatibility of the cements[54]. Fridland already proved that $Bi_2O_3$ was chemically inert[21]. Yun Chan et al also concluded that PC + $Bi_2O_3$ exhibits similar tissue reaction as MTA[63].

Group II (MTA) and Group IV (WPC + Iodoform) showed similar tissue reactions and no significant difference between them in all experimental periods. Previous study[11] proved that PC + Iodoform showed similar inflammatory reaction when compared to MTA. Iodoform has been successfully used in paste form mixed with $Ca(OH)_2$ in root canal treatment for infected primary teeth[57]. Because of its good radiopacity, similar tissue reaction and anti-inflammatory property, iodoform can be considered as an alternative to bismuth oxide.

Group II (MTA) and Group V(WPC + $ZrO_2$) exhibits similar inflammatory reaction in all the experimental groups. No previous study was noted as per pubmed search, incorporating $ZrO_2$ in Portland cement and tissue reaction was evaluated. This study concluded no significant difference between these two groups. Various reasons are there to consider $ZrO_2$ as an alternative radiopacifier to bismuth oxide.

Ceramic posts made from zirconium dioxide were used as aesthetic posts in post endodontic treatment of fractured anterior teeth[28]. Zirconium dioxide implants are supposed to be the wave of future. This material is attractive

because of its extraordinary properties such as high flexural strength (in excess of 1,000 MPa), hardness (1,200 – 1,400 Vickers). However, it is not only very strong, it is also biocompatible so that zirconium dioxide is also used in medicine (hearing devices and artificial fingers and hips) and dentistry (pins, crowns, bridges and implants). The fact that zirconium dioxide has the same colour as teeth along with its biotechnical characteristics mean it is used for manufacturing biocompatible, high-quality and aesthetic tooth and implant reconstructions.

In this study, formation of fibrous capsule was detected in all the groups. In the 7 day experimental period, the fibrous capsule was very thin. But at the end of 60 days after surgical procedure all groups exhibit thicker fibrous capsule formation. The observation was similar to previous studies.[11,47,31,63]

Mineral trioxide aggregate (MTA) has been shown to have good chemical and biological properties and its behavior has been extensively investigated in several clinical applications. The elevated cost of this product, however, has not allowed its use in all levels of health attention. According to the result of the present study white Portland cement mixed with radiopacifying agents (Bismuth oxide/Iodoform/Zirconium dioxide) has the potential to be developed as a root end filling material. WPC which is cheaper can replace MTA, so that cost is not factor in determining the treatment. However, some modifications to the material and subsequent extensive tests will need to be conducted to ensure that

the resultant material meets the medical device requirement. Methods to improve the setting time and compressive strength may also be explored that may lead to expanded clinical application of Portland cement, including direct pulp capping, indirect pulp capping, perforation repair, apexification, in addition to root end filling material.

## SUMMARY

The study was approved by the Ethical Committee, Tamil Nadu Government Dental College, Chennai and by the Animal Ethical Committee, Madras Medical College, Chennai-3. 18 albino rats were used in this study, divided into 3 sets of 6 animals each with respective to the experimental periods 7 days, 30 days and 60 days. 80wt%White Portland cement (Birla white) was mixed separately with 20wt% radiopacifying agents - Bismuth oxide, Iodoform, and Zirconium dioxide. All these three groups were compared with MTA (Pro Root) for tissue reaction in albino rat.

**EXPERIMENTAL GROUPS:**

GROUP I - EMPTY POLYETHYLENE TUBE

GROUP II - MTA

GROUP III - WHITE PORTLAND CEMENT 80wt% + BISMUTHOXIDE 20wt%

GROUP IV - WHITE PORTLAND CEMENT 80wt% + IODOFORM 20wt%

GROUP V - WHITE PORTLAND CEMENT 80wt% + ZIRCONIUM DIOXIDE 20wt%

All the test materials including MTA were mixed with sterile saline with water powder ratio of 3:1, and were loaded in to the polyethylene tube that were 1.2 mm of inner diameter and 5 mm length. After animal was anaesthetized, dorsal skin was shaved. Five incisions were made on the animals back with 0.5

cm length and with 2 cms apart. Five surgical cavities were created. Each of the polyethylene tube that were loaded previously with the respective test materials were placed into the surgical cavities. An empty polyethylene tube served as a control. After the respective experimental periods, 7 days, 30 and 60 days the animals were sacrificed and the tube along with the surrounding tissue were removed in block and sent for light microscopic histological analysis.

The inflammatory score (qualitative data) was analysed using Pearson Chi Square test. Each group was compared individually with each other.

In 7 days there was no significant difference between the groups. In 30 and 60 days all the test materials including MTA showed significant difference from the control (empty tube). But there was no difference between MTA and WPC mixed with radiopacifying agents – Bismuth oxide/Iodoform/Zirconium dioxide in all experimental groups.

# CONCLUSION

The results of the study conclude that the tissue reaction of the tested materials, white Portland cement (WPC) + Bismuth oxide, WPC + Iodoform and WPC + Zirconium dioxide were similar to MTA(Pro Root MTA) in all experimental periods7 days, 30 days and 60 days. But all these materials showed more inflammatory response than the control (empty tube) in both 30 and 60 days.

The result from our study support the idea that Portland cement has the potential to be used in clinical situations similar to those in which MTA is being used. Such an inexpensive and easily available material could allow very successful pulp treatments in many indigent patient population.

Nevertheless, other studies with different methodological models, increased sample number and experimental periods are necessary to guarantee the clinical applicability of Portland cement.

# BIBILOGRAPHY


1. **Aeinehchi M, Eslami B, Ghanbariha M, Saffar AS.** Mineral trioxide aggregate (MTA) and calcium hydroxide as pulp-capping agents in human teeth: a preliminary report. Int Endod Journal 2003;36:225-31.
2. **Amir Shayegan, Michael Petein, Astrid Vanden Abbeele.** The use of beta tricalcium phosphate, white MTA, white Portland cement and calcium hydroxide for direct pulp capping of primary pig teeth. Dental Traumatology 2009;25:413-19.
3. **Antonio Vinicius Holanda Barbosa, Gerhilde Callou Sampaio, Fabio Almedia Gomes, Daniel Pinto de Oliveira, Diana Santana de Albuquerque,Ana Paula Veras Sobral,** Short term analysis of human dental pulps after direct pulp capping with Portland cement. The Open Dentistry Journal, 2009,3,31-45.
4. **Ahmed Abdel Rahman Hashem, Ehab Hassenin.** ProRoot MTA, MTA-Angelus and IRM Used to Repair Large Furcation Perforations: Sealability Study. J Endod 2008;34:59–61
5. **Augusto Bodanezi, Nara Carvalho, Daniela Silva, Norberti Bernardineli, Clovis Monteiro Bramante, Roberto Brandao Garcia, Ivaldo Gomes de Moraes,** Immediate and delayed solubility of mineral trioxide aggregate and Portland cement. J Appl Oral Sci. 2008; 16(2):127-31

6. **Berit Olsson, Anthony Sliwkowski, Kaare Langeland,** Subcutaneous implantation for the biological evaluation of endodontic materials. J Endod 1981;7:355-69.
7. **Bramante Clovis Monterio, Ana Claudia Cardoso, Ivaldo Gomes de Moraes, Norberti Bernadineli,** Presence of arsenic in different types of MTA and white and gray Portland cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;106: 909-13.
8. **Bye GC (1999)** – Portland cement composition, production and properties 2nd edn. U.K. Thomas Telford Limited.
9. **Camilleri** J , Hydration mechanism of mineral trioxide aggregate, Int Endod Journal 2007:40:462-470.
10. **Carlos Eduardo de Silveira Bueno, Eduardo Gregatto Zeverino, Luiz Roberto Coutinho Manhaes, Daniel Guimaraes Pedro Rocha, Rodrigo Sanches Cunha, Alexandre Sigrist De Martin,** Study of bismuth oxide concentration required to provide Portland cement with adequate radiopacity for endodontic use. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;107:e65-e69.
11. **Carlos Alberto Herrero de Morais, Norberti Bernardineli, Roberto Garcia, Marco Duarte, Danilo Guerisoli,** Evaluation of tissue response to MTA and Portland cement with iodoform. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;102:417-21.

12. **Calvin D Torneck**, Reaction of rat connective tissue to polyethylene tube implants part I. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1966;21:379-88.
13. **Calvin D Torneck,** Reaction of rat connective tissue to polyethylene tube implants Part II. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1967 Vol.24, No.5.674-684.
14. **Camilleri J.** Evaluation of the physical properties of an endodontic Portland cement incorporating alternative radiopacifiers used as root end filling material, Int. Endod Journal, 43, 231-240: 2010.
15. **Coomaraswamy S Kristian, Philip J Lumley, Michael Hofmann,** Effect of bismuth oxide radioopacifier content on the material properties of an endodontic Portland cement based (MTA like ) system. J Endod 2007;33:295-98.
16. **De Deus, Audi C, Murad C**, Similar expression of through and through fluid movement along orthograde apical plus of MTA and white Portland cement. Int. Endod Journal 2008;41:1047-53.
17. **Daniel Araki Ribeiro, Marco Antonio Hungaro Durate, Mariza Akemi Matsumoto, Mariangela Esther Alencar Marques, Daisy Maria Favero Salvadori.** Biocompatability in vitro tests of MTA and regular and white Portland cements. J Endod 2005;31:605-9.
18. **Dennis C. Smith ,** Characteristics of dental tissues and their response to dental materials. , Biocompatability of dental materials, Volume I.1982

19. **Durate, Demarchi, Yamashita.** pH and calcium ion release of two root end filling materials. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;95:345-347.
20. **Eng Tiong Koh, Fraser Mc Donald, Pitt Ford, Mahmoud Torabinejad,** Cellular response to MTA. J Endod 1998 24:8;543-47.
21. **Fridland, Rosado,** MTA solubility and porosity with different water to powder ratio. J Endod 2003; 29: 814-17.
22. **Hamid Reza, Shahner Shah, Saeed Rahimi et al,** Connective tissue reaction to white and gray MTA mixed with distilled water or chlorhexidine in rats. Iranian Endodontic Journal 2005
23. **Howard Roberts, Jeffrey Toth, David Berzins, et al** . Mineral trioxide aggregate material use in endodontic treatment. A review of the literature Dent Materials 24 (2008) 149-164.
24. **Hakki S, Bozkurt SB, Hakki EE, Belli S.** Effects of Mineral Trioxide Aggregate on Cell Survival, gene expression associated with mineralized tissues, and biomineralization of cementoblasts. J Endod 2009;35:513-19.
25. **Holland R, de Souza V, Nery MJ, Otoboni Filho JA, Bernabe PF, Dezan Junior E.** Reaction of rat connective tissue to implanted dentin tubes filled with mineral trioxide aggregate or calcium hydroxide. J Endod 1999;25(3):161-6.

26. **Islam. I, H. K. Chng & A. U. J. Yap,** X – ray diffraction analysis of mineral trioxide aggregate and Portland cement. Int Endod Journal 2006;39:220-25.
27. **Islam, Hu Cheng, Adrian et al.** Comparison of physical and mechanical properties of MTA and Portland cement. J Endod 2006;32:193-98.
28. **Ingle's Endodontics** 5th edition.
29. **James, Simon, Samuel, Rafel et al.** Sterilization of Portland cement. Endodontology : 47 – 49.
30. **Joao Eduardo, Simone Watanabe, Pedro Felicio, Mariana Teixeira.** A mineral trioxide aggregate sealer stimulated mineralization. J Endod 2009;35:256-60.
31. **Jacob Saidon, Jianing He, Qiang Zhu, Kamran Safavi, Larz S. W. Spangberg,** Cell and tissue reactions to mineral trioxide aggregate and Portland cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;95:483-9.
32. **Katz A, Kaffe, Littner M** et al, Densito metric measurement of radiopacity of guttapercha cones and root dentin. J Endod 1990;16:211-3.
33. **Langeland K, James Gutiuso, Leena Langeland, Gabriel Tobon,** Methods in the study of biologic responses to endodontic materials. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1969;27:522-542.

34. **Marilia Gerhardt de Oliveira, Cristina Braga Xavier, Flavio Fernando Demarco, Antonio Luis Barbosa** Comparative chemical study of MTA and Portland cements. Braz Dent J (2007) 18(1): 3-7.
35. **Mohmoud Torabinejad, Hong, Mc Donald, Pitt Ford,** Physical and chemical properties of a New Root end filling material. J Endod 1995 21:7;349-53.
36. **Mohmoud Torabinejad, Chan Ui Hong, Pitt Ford, Stalin Kariyawasam,** Tissue reaction to implanted Super EBA and Mineral Trioxide Aggregate in the Mandible of Guinea Pigs: A preliminary report. J Endod 1995 21:11;569-71.
37. **Mohmoud Torabinejad, Petra Wilder Smith, DrMedDent, James Kettering, Pitt Ford,** Comparative investigation of Marginal Adaptation of Mineral Trioxide Aggregate and other commonly used Root End filling materials. J Endod 1995 21:6;295-99.
38. **Berit Olsson, Anthony Sliwkowski, Kaare Langeland,** Subcutaneous implantation for the biological evaluation of endodontic materials. J Endod 1981;7:355-69.
39. **Marco Antonio Hungaro Durate, Guaniara de Oliveira, Rodrigo Ricci Vivan, Juliane Maria Guerreiro Tanomaru, Mario Tanomaru Filho,** Radiopacity of Portland cement associated with different radiopacifying agents. J Endod 2009;35:737-40.

40. **Marilia Gerhardt, Cristina Braga, Flavio Fernando, Antonio Luis Barbosa, Anline Tempel, Daniel Humberto.** Comparative chemical study of MTA and Portland cements. Braz Dent J 2007;18:3-7.
41. **Mario Tanomaru, Juliane Tanomaru, Danilo Barros, Evandro Watanabe, Izabel Ito.** In vitro antimicrobial activity of endodontic sealers, MTA based cements and Portland cements. Journal of Oral Science,2007, Vol.49, No.1,41-45.
42. **Makkes PC, Van Velzen, Wesselink PR,** Polyethylene tubes as a model for the root canal. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1977;44:293-300.
43. **Ozbas H, Yaltirik M, Bilgic B, Issever H.** Reactions of connective tissue to compomers, composite and amalgam root end filling materials. Int Endod J 2003;36:281-87.
44. **Peng L, Ye L, Tan H, Zhou X.** Evaluation of the formocresol versus mineral trioxide aggregate primary molar pulpotomy: a metaanalysis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006; 102:40–44
45. **Razmi H, Zarrabian. M, Sharifian, Sharifi, Sasani, N. Ramezankhani,** A histologic evaluation on tissue reaction to three implanted materials (MTA, Root MTA, and Portland cement type I) in the mandible of cats. Journal of dentistry, Iran, 2004: vol.1, No.3:62-69.
46. **Renato Menezes, Clovis Monterio Bramante, Ariadne Letra, Vanessa Graciela Gomes Carvalho, Roberto Brandao Garcia,** Histologic

evaluation of pulpotomies in dog using two types of mineral trioxide aggregate and regular and white Portland cements as wound dressings. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;98:376-9.

47. **Ricardo Martinez, Maria Laura, Gabriel Kokubu**. Rat subcutaneous tissue response to Modified Portland Cement, a New Mineral Trioxide Aggregate. Braz Dent J (2009) 20(2): 112-117.
48. **Roberto Kochenborger, Fabiana Soares, Elaine Vianna.** Analysis of tissue reactions to methacrylate resin based, epoxy resin based and zinc oxide eugenol endodontic sealers. J Endod 2009;35: 229-232.
49. **Saliba E, S Abbassi-Ghadi, R Vowles, J Camilleri, S Hooper.** Evaluation of the strength and radiopacity of Portland cement with varying additions of bismuth oxide. IEJ 2009,42:322-38.
50. **Sema S. Hakki, S. Buket Bozkurt, Erdogan Hakki, Sema Bellit.** Effects of Mineral Trioxide Aggregate on cell survival, biomineralisation of cementoblasts. J Endod 2009 ;35:513-19
51. **Sarkar NM, Caicedo R, Ritwik P.** Physiocochemical basis of the biologic properties of mineral trioxide aggregate. J Endod 2005;31:97-100.
52. **Saeed Asgary, Masoud Parirokh, Mohammad Jafar Eghbal, Frank Brink**, Chemical differences between white and gray mineral trioxide aggregate, J Endod 2005;31:101-103

53. **Taisa Regina Conti, Vivien Thiemy, Ana Paula Camolese, Ana Beatriz Silveira, Thais Marchini Oliveria, Natalino Lourenco Neto, Maria Aparecida Andrade Moreira.**Pulpotomies with Portland cement in human primary molars. J Appl Oral Sci. 2009;17(1):66-9.

54. **Tauby Coutinho Filho, Gustavo De Deus, Leila Klein, Gisele Manera, Carla Peixoto, Eduardo Diogo Gurgel Filho,** Radiopacity and histological assessment of Portland cement plus bismuth oxide. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;106:e69-e77.

55. **Torabinejad M,Watson T, Pitt Ford TR,** Sealing ability of a Mineral Trioxide aggregate when used as root end filling material. J Endod 1993;19:591-96.

56. **Taylor HFW;** Cement chemistry. London; Thomas Telford; 1997;P113-225.

57. **Thomas AM, Chandra, Pandey K,** Elimination of infection in pulpectomized deciduous teeth: a short term study using a iodoform paste. J Endod 1994; 20: 253-5.

58. **Witherspoon E David, Joel C Small, John D Regan, Martha Nunn,** Retrospective analysis of open apex obturated with mineral trioxide aggregate. J Endod 2008; 34: 1171-76.

59. **William Philip Saunders,** A prospective clinical study of Periradicular surgery using Mineral Trioxide aggregate as a root end filling. J Endod 2008;34:660-665.

60. **Wixsen SK, White et al .** A comparison of Phenobarbitol, Fentanyl – Droperidol, Ketamine Xylazine & Ketamin-Diazepam anaesthesia in rats. Laboratory Animal Anaesthesia 37;6:726-30(1987).

61. **Yaltirik M, Ozbas H, Bilgic B.** Reactions of connective tissue to mineral trioxide aggregate and amalgam. J Endod 2004;30:95-9

62. **Yesiloy C, Koren LZ, Morse DR,** A comparative tissue toxicity evaluation of established and newer root canal sealers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1988;65:459-67

63. **Yun Chan Hwang, In Nam Hwang, Min Seok Kim, Ho Hyun Son,** Chemical composition, radiopacity, and biocompatibility of Portland cement with bismuth oxide. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;107:e96-e102.

64. **Zmener O.** Tissue response to a new methacrylate – based root canal sealer. Preliminary observations in the subcutaneous connective tissue of rats. J Endod 2004;30:348-51.